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ADMINISTRATION OF THE NATIONAL CANCER INSTITUTE ACT, AUGUST 5, 1937, to JUNE 30, 1943

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The National Cancer Institute Act, signed by the President on August 5, 1937, was recognition by Congress of the fact that greater efforts should be made to combat the ever-increasing mortality from cancer in the United States. Many factors have contributed to lengthening the span of life and to the gradual shift in the age groups of our population so that more and more men and women are living to reach the cancer age. By 1936 the mortality from cancer had reached the point where it accounted for approximately 1 death in every 10. The fact that the name of every member of the United States Senate was signed to the bill introducing the National Cancer Institute Act is evidence of the public consciousness of this problem. The act created in the Public Health Service a division called the National Cancer Institute, to be devoted exclusively to work which it was hoped would result in improving the treatment for cancer and in lowering the death rate from this disease.

The law states that the purposes of the Institute are—

> conducting researches, investigations, experiments, and studies relating to the cause, diagnosis, and treatment of cancer; assisting and fostering similar research activities by other agencies, public and private; and promoting the coordination of all such researches and activities and the useful application of their results, with a view to the development and prompt widespread use of the most effective methods of prevention, diagnosis and treatment of cancer.

The Institute was organized as a division of the National Institute of Health, of which Dr. L. R. Thompson was director. Dr. Carl Voegtlin was appointed the first chief.

Since this law was the first national effort in this country in the field of cancer, the administrators responsible for initiating the work had no model to guide them other than the law itself which, while specific on many points, was still general enough in its terms to allow wide latitude in the Institute's activities.

APPROPRIATIONS

The appropriations for the work of the Institute from August 1937 to June 30, 1943, have been as follows:

Fiscal year ended June 30:	*Appropriation*
1938	$400,000
1939	400,000
1940	570,000
1941	570,000
1942	565,000
1943	[1] 534,870

[1] Exclusive of funds for printing and travel.

NATIONAL ADVISORY CANCER COUNCIL

One of the first steps in the organization of the work of the Institute was the appointment of the members of the National Advisory Cancer Council, an advisory body created by the act, which consists of six members with the Surgeon General as chairman ex officio. The Surgeon General cooperates with the Council in carrying out the powers

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and duties imposed by the act. The law provided that the first six members were to be appointed in groups of two for 1, 2, and 3 years, respectively. As each group completed their terms, their successors were to be appointed for 3-year terms. As a result of this provision two members of the Council are appointed each year, and a continuity of activity is provided which would not be possible if all members completed their terms at the same time. No member may succeed himself, but he may be reappointed after 1 year.

Members.—The first Council brought together some of the outstanding men in the cancer field as well as administrators and leaders in certain branches of science. They were Dr. James Ewing, distinguished pathologist and director of Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York City; Dr. Frances Carter Wood, director, Institute of Cancer Research, Columbia University; Dr. C. C. Little, managing director, American Society for the Control of Cancer; Dr. Ludvig Hektoen, noted pathologist, formerly professor of pathology at Rush Medical College and director of the McCormick Institute for the Study of Infectious Diseases, Chicago; Dr. James B. Conant, president, Harvard University; and Dr. Arthur H. Compton, professor of physics, University of Chicago.

Other men who have served on the Council up to the present time are Dr. James B. Murphy, director of cancer research, Rockefeller Institute for Medical Research, New York City; Dr. Mont R. Reid, professor of surgery, University of Cincinnati; Dr. Max Cutler, director, Chicago Tumor Institute; Dr. George M. Smith, professor of anatomy, Yale University School of Medicine; Dr. E. A. Doisy, professor of chemistry, St. Louis University School of Medicine; Dr. C. P. Rhoads, director, Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York City (following the retirement of Dr. Ewing); Dr. Waltman Walters, surgeon, Mayo Clinic and professor of surgery, University of Minnesota; Dr. Baird Hastings, professor of biological chemistry, Harvard University.

Up to June 30, 1943, two Council members, Dr. Arthur H. Compton and Dr. James B. Murphy, had been appointed for second terms of 3 years and Dr. Mont R. Reid [1] had been reappointed to fill the unexpired term of Dr. Waltman Walters, who went into the military service.

Meetings.—The Council held its first meeting on November 9, 1937; since that date it has met 22 times. Before the war, meetings were held every 3 months or more frequently if necessary, but since December 1941 the number of meetings has been decreased.

Advisory service.—The continuous work of the Council, of course, is its advice on the activities of the National Cancer Institute. At each meeting the Chief of the Institute reviews the Institute's work and presents problems for discussion and recommendation.

Committees.—In addition to attending meetings, Council members have served on committees appointed to consider and advise the whole Council or the Institute on specific problems such as the training of physicians in the diagnosis and treatment of cancer, the purchase of radium, the development of cyclotron projects, study of genetics in relation to cancer, cancer nomenclature and records, cooperation in cancer research, and certain applications for grants-in-aid.

Conferences.—The Council has sponsored two conferences. The first one, held December 13, 1937, was a conference of administrators of cancer funds and directors of cancer research, the purpose of which was to give these leaders in the cancer field an opportunity to participate in the development of the activities proposed under the National Cancer Institute Act and to secure the benefit of their opinions and advice. The second conference, held October 11 and 12, 1940, dealt with gastric cancer and had as its object the stimulation of

[1] Deceased May 1943 and was succeeded by Dr. J. Shelton Horsley, Richmond, Va., appointed December 1, 1943.

interest in research on this important problem. This conference was attended by representatives of a large number of institutions with research departments. The proceedings of this conference were published in the Journal of the National Cancer Institute (vol. 1, No. 4, February 1941). A third conference was initiated by the Council through its Committee on Cooperation in Cancer Research but was held under the auspices of the International Cancer Research Foundation. This conference dealt with the endocrine aspects of cancer research and was held in Atlantic City June 5 and 6, 1942.

Two committees of the Council have also held conferences, one on special training in the diagnosis and treatment of cancer held in New York June 8, 1938, and the other on cancer nomenclature and records held in Washington February 25, 1939.

Committee on Cooperation in Cancer Research.—This committee sponsored the conference on gastric cancer. Following the conference, the committee enlisted the cooperation of several institutions which were interested in carrying on gastric-cancer research. Several research projects were initiated, some to be aided by grants from the National Cancer Institute and others to be supported by funds from other sources.

In an effort to stimulate earlier diagnosis of gastric cancer and thereby raise the percentage of 5-year cures, an article entitled "Problems of Gastric Cancer" was prepared at the Council's request by Dr. William A. Cooper, a research fellow of the National Cancer Institute, working at Cornell University Medical College. The paper was published in the Journal of the American Medical Association (vol. 116, No. 9, May 10, 1941).

Other plans of the Council to sponsor an educational campaign on gastric cancer both among the medical profession and the laity were interrupted by the war.

In an effort to promote the coordination of cancer research in the United States, the committee arranged for an exchange of information on activities between the Council and leading foundations supporting cancer research. A summary of the more important actions taken by the Council at its meetings is sent to each of the cooperating foundations.

Grants-in-Aid.—One of the important duties of the Council, as outlined in the National Cancer Institute Act, is to review applications for grants-in-aid and to make recommendations to the Surgeon General for grants in support of research projects which in its opinion show promise of making valuable contributions to human knowledge with respect to the cause, prevention, or methods of diagnosis or treatment of cancer.

The principles to be followed in recommending grants-in-aid were discussed by the Council at its first meeting. At the second meeting the following policy was adopted before any applications for grants-in-aid were considered.

(1) Grants-in-aid may be approved for such salaries and wages, such supplies and expenses, and the purchase or construction of such equipment and apparatus as may seem essential to conduct research that promises to result directly or indirectly in valuable contributions to the knowledge of cancer.

(2) The Council need not fix any limit to the amount of money it may recommend in a given case but will be governed by the nature and needs of the case and the amount of available funds.

(3) Applications for grants-in-aid shall be accompanied by budgets showing how the money requested is to be expended. Deviations from authorized budgets are not permissible without the previous approval of the Surgeon General. Grants-in-aid are subject to accounting as the Surgeon General may direct.

(4) The Council, wishing to conserve the funds made available by Congress for the purpose of cancer research to the end that they may be employed to the best advantage of the country and having in mind the somewhat unsatisfactory experience of other bodies in connection with the too widespread awarding of small grants over a wide-flung field, and further recognizing that many aspects of cancer research cannot be distinguished from research in pure biology which may be outside the interest or responsibility of the Government; having in mind all these considerations, the Council resolves that, during the present fiscal

year, grants-in-aid will be made only in the case of projects closely connected with the work of the National Cancer Institute, with the exception of projects designed to improve the radiation therapy, including the study of new methods of producing radioactive substances.

Although this policy referred specifically to the first fiscal year of the Council's work, it was subsequently reaffirmed and used as a guide in considering applications for grants-in-aid until January 19, 1942, when a revised policy was adopted. In the revision the statement with regard to approval of projects closely related to the work of the National Cancer Institute was amended to read—

that as the rule but without mandatory restriction preference will be given to applications for aid of projects related to the general program for investigation being developed by the National Cancer Institute and the Council;

and the following additional principles which were already being followed were incorporated:

In all cases the Council will give close consideration to the relative significance of projects proposed for grant-aided research, to the competence of the workers in question, and to the fitness of the centers concerned for research in the cancer field.

The Council may refer applications to special committees for consideration and report with recommendation before final action is taken.

The Council will not favor applications for small grants in aid of research under detached or isolated conditions, except in special cases.

The Council desires to avoid undue competition and unnecessary duplication in cancer research, clinical as well as experimental, and will endeavor to initiate worthy projects with a view to organizing active cooperation in research with other suitable agencies.

In 1939 a patent policy recommended by a committee composed of representatives from the National Advisory Health Council and the National Advisory Cancer Council was approved by the Council and was incorporated in the agreement which is signed by applicants for grants-in-aid under the National Cancer Institute Act. Under the terms of this policy the applicant agrees that if any patentable discoveries or inventions are made in the course of the work aided by any grant received as a result of the application, the applicant will, in consideration of such grant, refer to the Surgeon General of the Public Health Service for determination, the question of whether such patentable discoveries or inventions shall be patented and the manner of obtaining and disposing of the proposed patents in order to protect the public interest.

Up to July 1, 1943, 213 applications have been before the Council. Sixty-five grants-in-aid totaling $425,723.50 have been recommended and paid. A list of these grants is given herein. In some cases it has been necessary for the Council to appoint a committee to look into the merits of a particular application and even to visit the applicant institution in order to confer with the research workers who would be engaged on the project before making its recommendations.

The administrative work involved in making the grants and supervising expenditures is handled by the Institute in cooperation with the executive director of the Council. Grantees are required to submit quarterly financial and periodic progress reports.

RADIUM LOANS

Under section 5(a) of the National Cancer Institute Act radium may be purchased both for the use of the Institute and for loans to other institutions for "the study of the cause, prevention, or methods of diagnosis or treatment of cancer, or for the treatment of cancer." In making the first appropriation of $400,000 for the Institute, Congress provided that $200,000 of the amount should be used for the purchase of radium. Since these funds were available only until June 30, 1938, and since it was also important to give cancer patients the benefit of the radium as soon as possible, the carrying out of section 5(a) of the Act became a major consideration in the first year of the Institute's work. The subject was taken up at the first meeting of the National Advisory Cancer Council, and a committee was appointed to make recommendations concerning the radium purchase.

GRANTS-IN-AID CERTIFIED FOR PAYMENT FROM AUGUST 5, 1937 TO JUNE 30, 1943

Grantee	*Date paid*	*Amount*	*Project*
American College of Surgeons, Chicago.	Aug. 19, 1938	$6,600	Study of hospitals and clinics to determine what should be done further to meet needs for adequate clinical cancer service.
	June 8, 1939	7,500	
	Aug. 29, 1940	7,900	
	Jan. 20, 1942	7,500	
Barnard Free Skin and Cancer Hospital, St. Louis.	Aug. 9, 1939	5,000	Integration of changes in experimental carcinogenesis.
	July 8, 1940	5,000	
	July 9, 1941	5,000	
	July 1, 1942	5,000	
	July 19, 1943	5,000	
Bittner, Dr. John J., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.	Mar. 31, 1938	3,200	Relation of nursing to breast cancer in mice.
	Aug. 16, 1938	4,200	
	July 20, 1939	6,960	
California, University of, Berkeley, Calif.	May 2, 1938	30,000	Development of cyclotron for clinical research on therapeutic uses of neutron rays and artificial radioactive substances.
	June 8, 1939	23,000	Clinical investigation of cancer therapy with neutron ray.
	Jan. 25, 1940	1,800	Nature and amount of urinary gonadotropic hormone coincidental with various testicular neoplasms.
	May 14, 1941	600	
	Aug. 13, 1940	21,770	Effect of fast neutrons on cancer in human beings.
	Nov. 19, 1940		
	July 23, 1941	9,750	
	Sept. 21, 1942	6,400	
Chicago Tumor Institute, Chicago.	Feb. 18, 1941	16,800	Radiotherapy of radioresistant forms of cancer of the mouth, pharynx, and larynx.
	Oct. 2, 1942	10,700	
Chicago, University of, Chicago.	Sept. 20, 1938	2,000	Purification of extracts of human gastric cancer for certain substances profoundly influencing gastric physiology in the experimental animal.
		2,000	Relation of hormones to ovarian cysts, benign and malignant.
		3,500	Research on human-cancer etiology through the study of cancerous and precancerous tissues for the presence of carcinogenic substances, and Extension of research work on sex hormones into the field of cancer research.
	June 7, 1940	3,500	To trace and fractionate carcinogenic substances from cancer tissue and urine of cancer patients.

Grantee	*Date paid*	*Amount*	*Project*
Cincinnati, University of, Cincinnati.	Sept. 7, 1938	3, 452. 50	Study of clinical material to improve the present methods of diagnosis and treatment of cancer.
	Jan. 11, 1940	2, 400	
	Feb. 18, 1941	2, 400	
	Apr. 26, 1940	4, 020	Gastric carcinoma and its relationship to chronic atrophic gastritis.
	Aug. 23, 1941	7, 796	
	July 19, 1943	5, 000	
	Apr. 2, 1942	2, 400	To improve the present methods of diagnosis and treatment of cancer and to study the relationship between chronic gastritis and gastric cancer.
Cornell University, New York City.	Mar. 8, 1939	11, 900	Tissue metabolism fundamental to cancer. The Pasteur reaction, Meyerhoff cycle, and intermediary oxidation in tumors.
	July 17, 1941	2, 700	Quantitative study by in vitro methods of the general nitrogen metabolism of the liver tumor in rats, induced with *p*-dimethylaminoazobenzene.
	Dec. 15, 1942	1, 800	
Duke University, Durham, N. C.	June 4, 1938	2, 625	Study of the properties of the papilloma virus protein and related materials.
	Jan. 11, 1940	1, 000	
Harvard University, Cambridge, Mass.	Apr. 5, 1938	27, 550	Synthesis of carcinogenic compounds for the purpose of studying the relation between the chemical structure of those compounds and their carcinogenic activity.
Institute of Cancer Research, Columbia University, New York City.	July 7, 1938	8, 500	Research into the scientific literature to make available to laboratory research workers information essential to the intelligent planning of their projects and to make available to clinicians information concerning new techniques in the treatment of cancer cases.
Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.	Mar. 31, 1938	9, 900	Research in genetics of cancer.
	May 18, 1940	18, 000	
	July 26, 1941	15, 000	
	June 17, 1942	12, 000	
	May 19, 1943	12, 000	
Louisiana State University, Baton Rouge, La.	Nov. 13, 1940	500	Photographic and art work in connection with cancer research.
Martland, Dr. Harrison, chief medical examiner, Essex County, City Hospital, Newark, N. J.	Aug. 16, 1938	1, 425	Continuation of the study of osteogenic sarcomas in radioactive persons (dial painters).
Meharry Medical College, Nashville, Tenn.	Dec. 29, 1938	1, 425	Establishment of an adequate follow-up system and records which may be available for scientific and statistical studies.
	Apr. 20, 1940	1, 100	Continuation of records and follow-up of tumor clinic work of George W. Hubbard Hospital.
	June 6, 1941	1, 100	
	June 17, 1942	1, 100	
	July 19, 1943	1, 100	

Grantee	*Date paid*	*Amount*	*Project*
Memorial Hospital, New York City.	Aug. 2, 1940	3,300	Experimental study of means for increasing the response of tumors to radiation.
	Sept. 12, 1941	5,000	Metabolic studies on patients with gastric cancer.
	June 26, 1943	5,000	
Michael Reese Hospital, Chicago.	Jan. 25, 1940	3,600	Significant factors in the initiation of induced and spontaneous tumors.
	Aug. 24, 1942	2,400	Relation between the caloric level of food intake and the incidence of tumor formation.
Michigan, University of, Ann Arbor, Mich.	Apr. 19, 1938	1,800	Biologic comparison of X-rays and neutrons.
National Research Council, Washington, D. C.	June 18, 1938	3,000	Cooperation with the American Registry of Pathology in the collection and study of tumors with a view to increasing knowledge of their nature, diagnosis, and treatment.
	Jan. 25, 1940	3,000	
	May 23, 1941	1,000	
Nebel, B. R., New York State Agricultural Experiment Station, Geneva, N. Y.	Sept. 7, 1938	1,000	Cytology and physiology of non-green plant-tissue cultures under action of phenanthrene.
Rochester, University of, Rochester, N. Y.	Feb. 14, 1942	4,900	Metabolism in gastrointestinal cancer.
Society of the New York Hospital, New York City.	Aug. 4, 1939	6,000	Researches in the early diagnosis of gastric cancer. The comparative value of clinical methods.
	Feb. 6, 1942	5,000	
Wallace, Dr. Edward, University of Cincinnati, Cincinnati.	Apr. 11, 1938	4,350	Relation of pituitary gland to cancer.
	Aug. 13, 1940	1,500	Investigation of effect of climatic temperature upon induced, inoculated, and spontaneous cancer in experimental animals and continued study of hormonal influences, especially that of the pituitary upon cancer.
Washington University, St. Louis.	Oct. 11, 1940	16,000	Exploration of application of the cyclotron and products to the treatment and study of cancer.

After thorough investigation and consultation with experts as to the type of radium preparation most in demand for hospital use, it was decided that the Institute should purchase 9½ gm. of radium, 1 gm. of which would be in solution, and the balance distributed in units as follows:

Number of units	*Type*	*Quantity of radium, milligrams*
100	Needles	1.0
300	Needles	2.0
750	Cells	3.33
180	Needles	5.0
55	Tubes	10.0
90	Needles	10.0
39	Tubes	15.0
95	Tubes	25.0

The orders for the radium were placed as soon as bids could be secured.

While work on the specifications and purchase of the radium was in progress, consideration was also given to formulating the regulations under which the loans would be made. The basic points that had to be considered were limitations on the use of the radium to the purposes specified in the National Cancer Institute Act, free use for patients, preference for patients in the lower income groups, protection of patients against injury, and protection of the Government against loss. Tentative drafts of the regulations were submitted

to a number of specially qualified persons and to the National Advisory Cancer Council for criticism and suggestion. As finally adopted, the regulations are embodied in a loan contract which is made between the Government and the borrowing hospital in which the borrower agrees:

(1) To be responsible for the safekeeping of the radium and containers and for the cost of replacement of any radium and containers lost or damaged from the time the radium is delivered to the transportation agency by the Government until it is returned by the borrowing hospital.

(2) To use the radium only at the institution to which it is lent, and for the study of the cause, prevention, or methods of diagnosis or treatment of cancer.

(3) To make no charge to patients for the use of radium. (This provision does not cover medical, nursing, or hospital fees.)

(4) To give preference in the use of radium to patients whose financial circumstances are such that they cannot, without depriving themselves of the necessities of life, pay from their own resources for the cost of the use of the radium.

(5) To permit the radium to be used for treatment purposes only by experts whose qualifications are the equivalent of the standards established for radiologists by the American Board of Radiology.

(6) To maintain a standard of protection against exposure to high-energy radiation that will protect personnel working in the institution from exposure to more than one roentgen per week.[2]

(7) To furnish such reports as the Government may require pertaining to the use of the radium.

(8) To pack and deliver the radium to a responsible agency for shipment when the contract is terminated.

(9) To pay all transportation charges involved in shipping the radium to the borrowing hospital and return to the National Cancer Institute.

Standards for shipping.—Previous to the time the National Cancer Institute began its radium-loan program, no standards relative to the packing of radium for shipment had been established. The need for standards was recognized, as it was well known that overexposure to radium will cause injury to the personnel handling it and that slight exposure will damage or ruin films which are shipped in the same car with it. In order that a standard of protection might be worked out to the satisfaction of all concerned, a conference was held at the National Institute of Health February 8, 1939, by representatives of the Railway Express Agency (the agency apt to handle most of the shipments), certain radium and film companies, the National Bureau of Standards, and the United States Public Health Service. The result of this conference was the formulation, on a scientific basis, of standards for the amount of lead screening required in shipping different quantities of radium according to the length of time the shipment would be in transit. These standards were adopted by the Railway Express Agency and published for the guidance of its agents and shippers. To conform to these standards the National Cancer Institute has special lead shipping containers made in three sizes, so designed that each of the smaller containers fits into the next larger one. The walls of the smallest container are 1½ inches thick, each of the others being ½ inch thick, so that the lead screening around the radium may be increased to 2 or 2½ inches if necessary. This thickness of lead is considered sufficient to protect films against damage from as much as 100 mg. of radium for 85 hours if the radium is placed at a distance of 10 feet from ordinary film and 15 feet from X-ray film. One hundred milligrams is the maximum quantity that is packed in any one lead container.

Testing.—As soon as the radium was delivered by the radium companies, test-

[2] This section was not in the first contracts but was added later and is now embodied in all contracts.

ing was begun by the National Bureau of Standards, which, through a cooperative arrangement with the Institute, acts as custodian for all radium not in use, keeping it in a specially built vault lined with 6 inches of lead to prevent radiation injury to personnel. The Bureau of Standards also packs all radium for shipment, tests radium when it is returned from a hospital, and tests new preparations purchased by hospitals to replace any that they have lost.

Distribution.—Approximately 2 gm. of the radium have been used by the Institute in research and in the cancer clinic at the United States Marine Hospital, Baltimore. The balance has been lent to hospitals throughout the country. The first radium was ready to go out in September 1938. Fifty-five loans have been made since the beginning of the radium-loan program. Contracts are made for 1 year at a time and are terminated or renewed at the end of the term, as circumstances warrant. Forty-nine loans were in effect as of June 30, 1943.

The chief factors considered in determining whether a hospital is eligible for a loan are the amount of radium available in the area in which the hospital is located, the quantity of radium owned by the hospital, the number of cancer patients using the hospital's facilities, the number of loans already made in that area, and the qualifications of the personnel. Every effort has been made to effect an equitable distribution of the radium in order to serve as many different sections of the country as possible.

A list of the hospitals which have had radium loans to date follows:

Albany Hospital, Albany, N. Y.
Binghamton City Hospital, Binghampton, N. Y.
Broadlawns General Hospital, Des Moines.
Bonfils Foundation Tumor Clinic, University of Colorado, Denver.
Barnard Free Skin and Cancer Hospital, St. Louis.
Baroness Erlanger Hospital, Chattanooga.
Baylor University Hospital, Dallas.
Charlotte Memorial Hospital, Charlotte, N. C.
City-County Hospital, LaGrange, Ga.
Cook County Hospital, Chicago.
Danbury Hospital, Danbury, Conn.
Duke University Hospital, Durham, N. C.
Elizabeth Steele Magee Hospital, Pittsburgh.
El Paso City-County Hospital, El Paso, Tex.
Emory University Hospital, Emory, Ga.
Grace Hospital, New Haven, Conn.
Greenville General Hospital, Greenville, S. C.
Hillman Hospital, Birmingham, Ala.
Indianapolis City Hospital, Indianapolis.
Johns Hopkins Hospital, Baltimore.
Kansas City Municipal Hospital, Kansas City, Mo.
Los Angeles County General Hospital, Los Angeles.
Mary Fletcher Hospital, Burlington, Vt.
Meadowbrook Hospital, Hempstead, N. Y.
Medical College of Virginia, Richmond, Va.
Mercy Hall Cancer Hospital, Detroit.
Misericordia Hospital, Philadelphia.
Missouri Cancer Commission (for the Ellis Fischel State Cancer Hospital, Columbia, Mo.).
Mountain State Memorial Hospital, Charleston, W. Va.
Newark City Hospital, Newark, N. J.
Nashville General Hospital, Nashville, Tenn.
Newark Beth Israel Hospital, Newark, N. J.
Norton Memorial Infirmary, Louisville.
Norwalk General Hospital, Norwalk, Conn.
New Britain General Hospital, New Britain, Conn.
North Carolina Baptist Hospital, Winston-Salem, N. C.
Protestant Deaconess Hospital, Evansville, Ind.
The Queen's Hospital, Honolulu, Territory of Hawaii.
Receiving Hospital, Detroit.
St. Francis Hospital, Hartford, Conn.
St. Luke's Hospital, Denver.
St. Joseph's Infirmary, Louisville.
Sedgwick County Tumor Clinic, Wichita, Kans.
Shreveport Charity Hospital, Shreveport, La.
Stamford Hospital, Stamford, Conn.
Strong Memorial Hospital, Rochester, N. Y.
Swedish Hospital, Seattle, Wash.
Tri-County Hospital, Orangeburg, S. C.
University of Maryland Hospital, Baltimore.
University of Nebraska Hospital, Omaha.
University of Michigan Hospital, Ann Arbor, Mich.
University of Virginia Tumor Clinic, University, Va.
Vassar Brothers Hospital, Poughkeepsie, N. Y.
Vermont State Cancer Commission, for use at the Rutland Hospital and the X-ray and Radium Institute, Rutland, Vt.
Worcester City Hospital, Worcester, Mass.

Care and Use.—As each loan is shipped, the borrower is sent instructions on storing and handling the radium in order to prevent injury to personnel using it or working near it, and to prevent damage to the radium preparations. The borrowing hospitals

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are visited at intervals to check on the care and use they are making of the radium. Annual reports received from the hospitals show that up to June 30, 1943, approximately 7,000 patients had had the benefit of treatment by the National Cancel Institute radium.

TRAINEESHIPS IN THE DIAGNOSIS AND TREATMENT OF CANCER

In recognition of the need for more physicians specially trained in the diagnosis and treatment of cancer, Congress included in the National Cancer Institute Act a provision authorizing the Surgeon General—

> To provide the necessary facilities where training and instruction may be given in all technical matters relating to diagnosis and treatment of cancer to such persons as in the opinion of the Surgeon General shall have proper technical training and shall be designated by him for such training or instruction * * *.

Ways and means of carrying out this section of the act were also taken up at the first meeting of the National Advisory Cancer Council, and Dr. James Ewing was appointed chairman of a group to study the question and make recommendations.

On January 8, 1938, a conference to consider the subject was held in New York City in which the following persons participated: Dr. C. Sidney Burwell, dean, Harvard Medical School; Dr. Stanhope Bayne-Jones, dean, Yale University School of Medicine; Dr. J. M. T. Finney, professor of surgery, emeritus, Johns Hopkins School of Medicine; Dr. Bowman C. Crowell, American College of Surgeons, Chicago; Dr. Burton T. Simpson, director, State Institute for the Study of Malignant Disease, Buffalo, N. Y.; Dr. Frank E. Adair, Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York City; Dr. Fred J. Hodges, professor of roentgenology, University of Michigan; Dr. John E. Wirth, director, Tumor Institute of the Swedish Hospital, Seattle; Dr. Charles Griffith, medical director, Veterans Administration, Washington, D. C.; Dr. Francis C. Wood, director, Institute of Cancer Research, Columbia University; Dr. Ludvig Hektoen, executive director, National Advisory Cancer Council; Dr. R. R. Spencer, executive assistant, National Cancer Institute.

The recommendations made at this conference were later considered by the Council and the Institute, and the training program was developed as fast as circumstances permitted. Certain medical centers where there was interest in cancer and where the staff was able to give suitable training to physicians wishing to prepare themselves for clinical cancer work were selected as training centers. As worked out, the plan provides for the appointment each year of a number of physicians carefully selected after personal interview who already have sufficient postgraduate training to prepare them for specialized work and who wish to devote themselves to a career in the field of clinical cancer. These trainees are appointed for 1 year at a time with the privilege of renewal for 2 additional years if their work is satisfactory both from the standpoint of their own performance and the quality of the instruction and experience provided by the training institution.

The usual stipend is $6.00 per day, but appointments may be and sometimes are made at higher rates of pay if circumstances warrant. The maximum stipend that may be given is $10.00 per day.

Twenty-eight hospitals have cooperated in this training program to date, the one making the largest contribution being Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York City, where more than 20 physicians have taken all or part of their training. The University of Minnesota Hospital has given training to 9 men; the University of Michigan Hospital, 7; and the Chicago Tumor Institute, 5. Twelve hospitals have had only 1 trainee use their facilities. Up to July 1, 1943, 56 physicians had received training under this program, 13 were in training on that date, and 3 were in process of being appointed. It was

planned to appoint others during the year. Of the 56 whose appointments had terminated, 24 had been in training 2 years or longer. Although a few physicians have terminated their training before the completion of 1 year, this has been chiefly for the purpose of entering military service; in only 1 case has an appointment been made of a person who wished to take less than 1 year's training.

The war has curtailed the training program both by reducing the number of physicians available for training and by calling into military service men who were in training.

The physicians who have finished this training and have not entered military service are filling important positions both in medical institutions and in private practice in various parts of the country.

It is believed that this training program will contribute in no small degree to making available to the cancer patient better facilities for the diagnosis and treatment of cancer.

RESEARCH FELLOWS

Section 5(c) of the National Cancer Institute Act authorizes the Surgeon General to establish and maintain fellowships in the Institute. Several of these fellowships were established in the first and second years of the Institute's existence in order to train personnel from which to draw the Institute's staff as soon as the new building was ready. Up to July 1, 1943, 40 persons, representing many different fields of science, have been given fellowships for varying periods of time; 9 research fellows were on duty as of that date, 2 physicians, 4 chemists, 1 geneticist, and 2 physicists.

JOURNAL OF THE NATIONAL CANCER INSTITUTE

One of the purposes of the National Cancer Institute, as stated in section 1 of the National Cancer Institute Act, is to promote the useful application of the results of research and investigations relating to the cause, diagnosis, and treatment of cancer. In order that this might be done it was necessary that the results of the work of the Institute be made readily available for the use of other workers in the cancer field. It was thought that a journal, issued at regular intervals, would be the most suitable way of making this material available and of preserving a permanent record of the Institute's work for future reference. Accordingly, permission to issue a bimonthly publication to be known as the Journal of the National Cancer Institute was secured from the Bureau of the Budget early in 1940 and the publication of the Journal was begun in August of that year. In addition to the reports of the work done at the Institute, reports of some of the studies supported by grants-in-aid and occasionally a review or special report by some leading investigator are published in this Journal.

The free edition of the Journal is limited to 1,000 copies which are sent to the leading scientific institutions in this country and to a number in foreign countries, particularly those in the Western Hemisphere, to all grade A medical schools, and to leading scientists working in the cancer field. Inasmuch as the edition of the Journal is too limited to supply all the persons interested in receiving it, arrangements have been made with the Superintendent of Documents, Washington, D. C., whereby subscriptions may be placed with him at the nominal price of $2.00 per year. Single copies may also be purchased from the same office for 40 cents each.

FUNDAMENTAL CANCER RESEARCH

Since control of cancer depends upon knowledge of its causes and methods of prevention, diagnosis, and treatment, the paramount activity of the National Cancer Institute is research on these problems. In order that the administrators of the Institute might have the benefit of the advice of experts from other research groups in planning this part of its work, the Surgeon General asked the following men to serve on a committee on fundamental cancer research "to formulate, as far as this could be done, the fundamental aspects of the cancer prob-

lem and to suggest various lines of work which merit investigation":

Dr. Stanhope Bayne-Jones, professor of bacteriology and dean of the school of medicine, Yale University.

Dr. Ross G. Harrison, chairman of the National Research Council, and Sterling professor of biology, Yale University.

Dr. Clarence C. Little, director, Roscoe B. Jackson Memorial Laboratory.

Dr. John Northrop, member, Rockefeller Institute for Medical Research.

Dr. James B. Murphy, member, Rockefeller Institute for Medical Research, chairman.

The committee's report (Pub. Health Rep., vol. 53, No. 48, December 2, 1939) which was submitted to the National Advisory Cancer Council July 25, 1938, was used as a guide in planning the work of the Institute.

Several years previous to the enactment of the National Cancer Institute Act, the Public Health Service had recognized the growing importance of cancer as a cause of death and had begun research work on the disease. The group of workers engaged in this research formed the nucleus of the research staff of the new Institute. It was more than 2 years from the time the act was passed until the specially designed and equipped building, for which the act provided, was ready for occupancy. As completed in 1939, the laboratories of the National Cancer Institute are outstanding in size and equipment. In the interim between the enactment of the law and the completion of the new building, the staff already working on cancer was augmented as far as existing laboratory facilities permitted and plans were made for bringing the staff up to full strength as soon as the new laboratory was available. The land on which the National Cancer Institute is built was given to the Government by a public-spirited citizen, Mrs. Luke Wilson, Bethesda, Md.

The research program as finally developed is a comprehensive one including projects in all of the more important basic medical sciences since, in the judgment of those people who have given the most consideration to the problem, ultimate success in the work can be attained only by coordinated research in many branches of science.

A staff of approximately 30 scientists with their assistants plan and carry on the research work on which more than half of the Institute's appropriation is spent. The entire scientific staff or small groups meet at frequent intervals for discussion and criticism of the work; each member of the staff is thus fully informed on what other staff members are doing and has the opportunity to contribute any ideas he may have to the work of his associates and in return to receive suggestions from them. Visiting scientists are invited to lecture to the staff.

Most of the laboratory research studies fall into six general categories:

(1) The experimental production of neoplasms.

(2) In vivo and in vitro studies of the mode of action of carcinogenic substances.

(3) Studies of the characteristics of cancerous tissue (the cytology of cancer and normal cells and the metabolic behavior and enzyme patterns of cancer cells contrasted with those of normal cells of origin).

(4) The role of dietary factors in the genesis and treatment of cancer.

(5) The role of hormones in the genesis and treatment of cancer.

(6) Experimental treatment of neoplasms in animals (in vivo and in vitro chemotherapy).

To those unfamiliar with cancer research work, it may be of interest to know that these studies require the use at all times of approximately 30,000 pure inbred mice, a large number of rats, and other animals such as guinea pigs, rabbits, dogs, and chickens. Only pure inbred mice whose genetic history with reference to cancer is known can be used in most of these studies. If mongrel strains of unknown genetic background are used, it may not be possible to determine which results are due to inherited characteristics and which are due to the treatment given the animal.

Those who recognize the complicated nature of cancer believe the solution of

the problem will not come as the result of one great discovery but rather by the piecing together of the many little discoveries made in the course of one research study after another. The work is slow, laborious, and often discouraging, but each new fact discovered adds to that beam of light which will eventually illuminate this "great darkness."

CLINICAL RESEARCH CENTER

The development of a tumor clinic in one of the United States Marine Hospitals, which serves the twofold purpose of providing expert care for beneficiaries of the United States Public Health Service and of supplying a clinical research center for the National Cancer Institute, is another important accomplishment under the National Cancer Institute Act. A committee composed of representatives of the Public Health Service and two physicians specially trained in cancer work drew up the plans for the establishment of this clinic which were considered by the National Advisory Cancer Council at its fourth meeting February 14, 1938, and approved in principle. The United States Marine Hospital at Baltimore was selected as the one most suitable for the location of the clinic since it was the nearest to the National Cancer Institute at Bethesda, Md. Space was provided by adding a new floor to one of the hospital wings. Construction work was begun soon after the plan was approved, and the clinic was ready for use late in 1939. Modern in every respect, it ranks with the best tumor clinics in the United States. A complete description of the clinic will be found in Public Health Reports (vol. 55, No. 48, November 29, 1940). Any cancer patient east of the Mississippi who is a beneficiary of the Public Health Service is eligible for admission to this clinic. Nearly 1,600 patients had been admitted up to July 1, 1943.

As progress is made in diagnostic methods and therapy, patients at this clinic will have the advantages of their use. The records of the clinic will be used for study and for evaluation of different types of therapy.

STATISTICAL STUDIES

Critical studies of cancer statistics are not only necessary for the planning of public health activities in the cancer field but may also be expected to provide new facts which will help point the way to laboratory research problems. Accordingly, a considerable portion of the Institute's appropriations has been spent on work connected with the analysis of available cancer statistics and in gathering new material. Special studies have been made of the available statistics on cancer mortality and the following reports published:

Trend of Recorded Cancer Mortality in the Death Registration States of 1900 from 1900 to 1935. Public Health Bulletin No. 248.

Recorded Cancer Mortality in Geographical Sections of the Death Registration States of 1920 from 1920 to 1935. Public Health Bulletin No. 252.

Geographic Variation in Recorded Cancer Mortality for Detailed Sites, for an Average of the Years 1930–1932. Public Health Bulletin No. 257.

Age Variation in Mortality from Cancer of Specific Sites, 1930–32. Public Health Bulletin No. 275.

Field studies have been undertaken to collect data on the incidence and epidemiology of cancer in representative sections of the United States and on the results of cancer therapy. This material is secured from physicians' and hospital records and by personal interviews with cancer patients. The following reports on cancer incidence have been published:

Incidence of Cancer in Atlanta, Ga., and Surrounding Counties. Public Health Reports 54: 1255–1273, July 14, 1939.

Incidence of Cancer in Cook County, Ill., 1937. Public Health Reports 55: 628–650, April 12, 1940.

Incidence of Cancer in Pittsburgh and Alleghany County, Pa., 1937. Public Health Reports 56: 708–739, April 4, 1941.

Incidence of Cancer in New Orleans, La., 1937. Public Health Reports 56: 1141–1170, May 30, 1941.

Incidence of Cancer in Philadelphia, Pa., 1938. Public Health Reports, 57: 1843–1857, December 4, 1942.

Incidence of Cancer in Dallas and Fort Worth, Texas, and Surrounding Counties, 1938. Public Health Reports, 57: 125–139, January 23, 1942.

Incidence of Cancer in Birmingham and Jefferson County, Alabama, 1938. Public Health Reports, 57: 377–397, March 13, 1942.

Incidence of Cancer in San Francisco and Alameda Counties, 1938. Public Health Reports, 57: 1566–1586, October 16, 1942.

Incidence of Cancer in Denver, Colorado, 1939. Public Health Reports 57: 1971–1986. December 25, 1942.

Incidence and Prevalence of Cancer of the Lung. Public Health Reports, 58: 1265–1271, August 20, 1943.

A consolidated report on the cancer incidence data was in process of preparation during the period covered by this review and was published subsequent thereto (Pub. Health Rep., vol. 59, Nos. 2, 3, and 4).

The collection of data in the epidemiological and therapeutic study is still going on. A preliminary report, Observations on the Familial Incidence of Cancer, based on data collected from 1,029 cancer patients, has been published (Am. J. Pub. Health, vol. 31, No. 1, January 1941) and another on the results of therapy of breast cancer appears in this issue of the Journal (pp. 445–460). Work on the analysis of data on cancer of certain sites, collected in these two studies, is under way.

EXAMINATION OF CANCER CURES

The Institute receives numerous letters from people who have, or who know someone who has, what they consider a "cancer cure." Most of these cures are formulas for a paste or a salve which can be used only for treatment of external cancer. These correspondents are told that formulas of this kind have been known to the medical profession for many years but that since the discovery of X-ray and radium they have had only a limited use, therefore there is nothing to be gained by testing other formulas of the same or similar composition.

As to the examination of other types of treatment a definite policy approved by the National Advisory Cancer Council has been adopted. This policy provides that the applicant must meet the following requirements:

(1) The method of treatment must be explained fully. There must be no secrecy whatsoever in regard to the composition or the nature of the treatment.

(2) Complete clinical records must be submitted of a suitable number of cancer patients treated with the remedy or method in question under competent medical supervision, and in each case the diagnosis of cancer must rest on competent and verifiable microscopic examination.

EDUCATION AND INFORMATION SERVICES

No small part of the Institute's work is in the field of public education and general information service. Individuals and organizations ask for information concerning cancer therapy, location of cancer specialists and clinics, statistics on cancer incidence and mortality, for literature for use with groups of various kinds such as clubs, public health nurses, and school children, for information concerning claims made for "cancer cures," and for material on State cancer laws and programs.

Very soon after the establishment of the Institute two educational projects were undertaken in cooperation with the American Society for the Control of Cancer:

(1) The production of a two-reel moving picture film "Choose to Live," designed to impress upon audiences the importance of seeking advice immediately when any of the well-known cancer danger signals are discovered. The film has now been shown in motion-picture houses all over the United States. Sixty-five copies have been purchased by outside organizations for local use. The Public Health Service has 17 copies which may be borrowed without cost to the borrower other than the payment of transportation charges.

(2) The publication of a folder "What To Know, What To Do About Cancer," 48,000 copies of which have now been distributed free and 216,000 copies have been sold by the Government Printing Office. Some of the main facts about cancer are presented in this folder in a brief form designed for quick reading, and the price of $1.00 per hundred copies (less if purchased in large

quantities) is so low that it is within the means of any organization.

Other literature of the popular type distributed by the Institute includes pamphlets entitled "What You Should Know About Cancer," "The Cures for Cancer," and "Cancer, a Discussion for Lay Audiences."

A set of posters on cancer has also been prepared for free distribution.

The Institute maintains a consulting service for State and local organizations interested in inaugurating cancer-control activities or in sponsoring cancer-control legislation. It has twice published in mimeographed form summaries of the cancer-control activities carried on by State cancer commissions and State departments of health, and a third paper on the subject was published in Public Health Reports (vol. 57, No. 43, October 23, 1942). Subsequent to the period included in the present review, another report has been prepared and was published in Journal of the National Cancer Institute (vol. 4, No. 4, February 1944). Mimeographed copies of State cancer-control laws are also kept current to send out in response to requests for information on this subject.

Many visitors come to the Institute both in groups and singly to see the work that is going on or to get information. These include research workers, physicians, nurses, teachers, students, club women, and plain John and Mary Citizen.

As far as possible, the Institute has complied with requests to send speakers to large meetings. This activity has necessarily been curtailed by the war but will be resumed when conditions are again normal.

Although it is too early in the Institute's history to speak of results, it is believed that the foregoing summary of activities presents a very creditable record. This is especially true when it is remembered that by the time the work of the Institute was fully organized this country was drawn into World War II. The inevitable loss of many staff members to the armed forces and to other activities connected with the war stopped some activities altogether and seriously interrupted or delayed others.

EVALUATION OF BREAST-CANCER THERAPY AS A GUIDE TO CONTROL PROGRAMS

By JAMES W. HAWKINS, *passed assistant surgeon, National Cancer Institute, National Institute of Health, United States Public Health*

Until the prevention of cancer becomes effective and practical on a broad public health scale, the control of the disease will remain dependent on cancer therapy. The tools of cancer therapy, surgery and high-energy radiation, have been known and used for many years, and it is accepted that their application must be specific not only for cancer of each part of the body but also for the various forms and stages of cancer. Dosage is important in X-ray therapy; yet prior to the adoption of the r unit by the Second International Congress of Radiology in 1928, there was no common unit by which dosage was measured. Numerous factors have been suggested as playing associated roles in cancer therapy. It has been difficult for individual physicians and hospitals to gain sufficient evidence to elucidate these factors. With these considerations, the National Cancer Institute in 1938 began a study of cancer therapy based on the combined experience of a group of hospitals.

SOURCE AND ORGANIZATION OF DATA

From the many hospitals which offered their cooperation in this program, the following [1] were selected: The State Institute for the Study of Malignant Disease, Buffalo; Pondville Hospital, Wrentham, Mass.; Palmer Memorial Hospital and Collis P. Huntington Memorial Hospital, Boston; Barnard Free Skin and Cancer Hospital, St. Louis; American Oncologic Hospital, Philadelphia; Albert Steiner Clinic for Cancer and Allied Diseases, Atlanta; University Hospital, Ann Arbor; and Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York.

A study period of from 2 to 6 years between January 1929 and January 1936 was selected in each hospital. Allowing for a follow-up period of 5 or more years, the case records of all patients who were first admitted during the study period, who were treated for cancer, and who were observed at least once after treatment were individually reviewed and summarized by trained representatives [2] of the National Cancer Institute. The records were then grouped according to the site of cancer.

This report deals exclusively with 3,105 cases of breast cancer. The classification of these cases by single and multiple primary lesions, by sex, and by color, is shown in table 1. Among those classified as multiple primary are included all cases of breast and other forms of cancer, regardless of which of the two or more cancers served as the basis of selection of the patient. Except in the discussion of secondary-attack rates, all subsequent data deal exclusively with single primary lesions.

TABLE 1.—*Persons with breast cancer, classified by single and multiple primary lesions, by sex and color*

Sex	Race	Primary cancers			
		Single	Multiple		Total
		Breast	Both breasts	Breast and others	
		Number	*Number*	*Number*	*Number*
Female	White	2,763	42	109	2,914
	Colored	153	2	4	159
Male	White	27	0	3	30
	Colored	2	0	0	2
Total		2,945	44	116	3,105

The ratio of males to females, 1 to 96, is about usual (*1*). The ratio of Negro to white, 1 to 18.3, is slightly below the ratio of deaths as reported for Negro

[1] The assistance given by these institutions is gratefully acknowledged.

[2] Virginia C. Tiffany and Bernard A. Koteen reviewed and summarized the case records.

and white in 1940 by the United States Bureau of the Census (*2*). The data covering males and Negroes are too small for independent analysis; and though the trends of these components will be given, except where otherwise indicated, the discussion will hinge on the relatively large and more homogeneous group of white females.

CRITERIA AND METHODS OF EVALUATION

To test the effectiveness of various types of treatment in breast cancer, it was deemed advisable not only to consider the variables in the forms of treatment, but also those within the host, the cancer process, and the methods of statistical evaluation. Although clinicians utilize the local response of the tumor and the general response of the patient in their evaluations, in the present paper the evaluation is confined principally to the status of the patient 5 years after first treatment. It must be emphasized that 5 years is not an end point but merely a period commonly used which is sufficiently removed from first treatment to permit tentative conclusions. Although in early forms of cancer treated with radical surgery, the annual mortality during the fifth year of follow-up approached that for the general population, in all other groups the mortality was such that prediction of end results based on the status of the patient at the beginning of the sixth year of follow-up appeared unwarranted.

Wide variations in reported cancer fatality rates frequently involve differences in statistical methods, the introduction of subjective rather than objective criteria, and, occasionally, unwarranted ultraconservatism or overoptimism. Ideally, the statistical method should consider each of the variables that alter the results, if such variables are unequally distributed throughout the methods of treatment under consideration, and if such variables remain uncorrected after consideration of the associated variables. An attempt is made herein to outline the most objective components of the material and the simplest methods of analysis.

Although the data as presented in tables 3, 4, and 10 are complete in that for those dead, lost, or living, the disease status is shown as present, absent, or unknown, such a method is unwieldy. No attempt is made, however, to define cures. Since so-called cure rates eventually involve assigning those dead without disease to cured, and those living with disease to failures, such cure rates may be misleading. Simple survival rates express the percentage of total patients who are alive, with or without evidence of having cancer. However, for groups with high survival (stage I, table 3) and for older age groups (table 10), the percentage of dead without disease is nearly the same as that for patients living with disease, and even for more advanced stages and for younger groups the tendency for balance persists. With both old age and early stage the percentage of those dead without disease is greater than that for those living with disease. In large groups the survival rates 5 years after first treatment based on known stage of disease are but slightly lower than theoretical cure rates in which living with disease is defined as failure and dead without disease as success. As table 3 shows, it is impractical to expect any form of treatment to prolong beyond 5 years the lives of many patients with advanced metastatic cancer. It is also conceivable that high-energy radiation might result in late deaths in the absence of cancer. It is frequently difficult to determine the status of the disease in living patients, especially those treated with X-ray. In the case of patients dying outside the hospital, it is also difficult to determine whether or not cancer was present and contributed to the death. It is relatively easy, however, to determine whether the patient is alive or dead. For these reasons, in summary presentations simple fatality rates have been used, and no attempt has been made to introduce corrections for those dying without cancer or those living with cancer.

The problems involved in handling lost cases [3] in summary presentations cannot be answered until complete follow-up is attained. Although some of the hospitals conducted nearly perfect follow-up, lost cases averaged 7.1 percent among white females and 32 percent among Negroes. A review of these lost cases revealed that they were broadly distributed in the 5-year period, and averaged between 2 and 3 years of follow-up. Groups with high survival naturally had a higher percentage of lost cases than did those with low survival. (See table 3.) Of more direct concern, however, is the fact that the average status of these lost cases, based on the presence or absence of disease at the time they were last observed, was as good as that of the traced cases at the same period of follow-up. Because, by definition, all these persons were living and since but 2 or 3 years remained of the 5-year period, the yearly mortality among the lost could have been twice that of the traced cases, without reducing the 5-year survival rates of the former to the level observed in the latter. For this reason, in these hospitals the exclusion of lost cases is considered a conservative method of analysis. This procedure probably introduces but little error in comparing white female groups having approximately similar survival rates and offers a more direct approximation of the theoretical rate than is obtained by considering lost cases as failures. In the calculation of yearly survival rates, lost cases were not excluded until the year in which they were lost.

VARIATIONS IN THE CANCER PROCESS

Variations in the cancer process refer to differences in the stage of disease at first treatment, in the histologic classification, in the duration of disease prior to first treatment, and in the criteria utilized in the diagnosis of cancer.

[3] The term "lost cases" refers to those patients who have not been followed as long as 5 years, and whose status 5 years after the initial treatment is consequently unknown.

STAGE OF DISEASE AT FIRST TREATMENT

A review of the various methods used in establishing the stage of disease at the time of first treatment revealed the fact that while 1 author may use as many as 12 different groups (*3*) another (*4*) may classify his patients merely as operable or inoperable. Obviously, the latter method offers no common basis for the comparison of persons treated with high-energy radiation and those treated with surgery, since the assumption is made that all early cases are operable. In general, metastatic involvement is more directly associated with the death of the patient than is the local process in the breast, and therefore a consideration of the presence or absence of regional and distant metastases is considered fundamental to proper classification.

In this study use has been made of all the available data from both the clinical and pathologic findings available at the first hospital admission. All patients with metastatic involvement of regions other than the axilla have been grouped in stage III, regardless of the presence or absence of axillary-node involvement. All the remaining cases with axillary metastases are termed "stage II," and the rest "stage I." Table 2 gives the sites involved in each stage and shows the number of patients by sex and color. Table 3 shows the 5-year follow-up of these patients by stage of disease. The survival rates of traced cases for stage I are 57, for stage II 23, and for stage III 3 percent. Thus the stage of disease at first treatment constitutes a major variable when tested in terms of 5-year survival rates. Stage IV, as here used, includes all patients for whom adequate description of the stage of the disease was not shown in the records and includes chiefly those treated in other hospitals prior to entering the hospitals selected. However, when patients were concurrently treated in the hospitals selected for this study and other hospitals, adequate descriptions were usually available, and these patients were classified in the first three stages.

TABLE 2.—*Stages of disease at first treatment of persons with single primary breast cancer, by sex and color*

Stage of disease	Site involved			Cases				
	Breast	Homolateral axillary nodes	Other metastases	Female		Male		Total
				White	Colored	White	Colored	
				Number	*Number*	*Number*	*Number*	*Number*
I	+			671	30	7	1	709
II	+	+		1,138	74	13	1	1,226
III	+	±	+	414	33	2		449
IV	+	(1)	(1)	540	16	5		561
Total				2,763	153	27	2	2,945

[1] Unspecified.

TABLE 3.—*White female patients with single primary breast cancer 5 years after first treatment, by stage of disease for all therapy*

Status of—		Stage at first treatment							
Patient	Disease	I		II		III		IV	
		Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Dead	Present	211	31.5	748	65.9	385	93.0	354	65.6
	Absent	41	6.1	56	4.9			11	2.0
	Unknown	6	.9	8	.7				
Total		258	38.5	812	71.5	385	93.0	365	67.6
Lost [1]	Present	17	2.5	43	3.8	15	3.6	17	3.1
	Absent	50	7.5	41	3.6	3	.7	3	.6
	Unknown	3	.4	2	.2			2	.4
Total		70	10.5	86	7.6	18	4.3	22	4.1
Living	Present	49	7.3	67	5.9	6	1.4	71	13.1
	Absent	286	42.8	161	14.2	5	1.2	72	13.3
	Unknown	6	.9	9	.8			10	1.9
Total		341	51.0	237	20.9	11	2.6	153	28.3
Grand total		669	100.0	1,135	100.0	414	100.0	540	100.0

[1] The term "lost" refers to patients whose follow-up was less than 5 years, and whose status 5 years after the initial treatment is therefore unknown.

In the general group referred to as stage I, at least three different methods are used in which an attempt is made to differentiate the very early cancers according to the size of the primary lesion and its location in the breast (*5*, *6*, *7*). Emphasis is placed by some writers (*8–11*) on the separation of late lesions on the basis of such findings as the presence of skin attachment, local ulceration or local nodules in the skin, attachment to deep tissues, diffuse or multiple lesions, edema of the breast, complete fixation to chest wall, and "inflammatory" carcinoma.

Although the descriptions of the local disease available for this study as a whole, did not permit a subclassification of all cases from early to late involvement among those in group I, there were sufficient cases to test some of these criteria. In all instances where the lesions are described as having a diameter of 1.5 cm. or less, the survival rate was 85.7 percent; the survival rate of patients with ulceration of the skin was 44 percent for all types of treatment; for those treated with radical surgery, the rate was 60 percent. On the other hand for the occasional cases, 5 in stage I, in which there was complete attachment to the breast wall, only 1 patient survived 5 years. Although only 1 of 15 patients with inflammatory carcinoma was in

stage I, all those with inflammatory carcinoma were dead by 5 years. Thus, with rare exceptions, the absence of axillary and distant metastases indicates good but not necessarily uniform progress. Of greater importance is the fact that 51 percent of the late cases in stage I had radical surgery, whereas for the series as a whole 57 percent of those in stage I were treated by this method. Thus variations in the extent of stage I cancer did not significantly shift the distribution of treatment by radical surgery.

Among 71 traced cases in which cancer was known to be primary in the inner quadrant, the survival rate was 52.1 percent for all types of therapy, whereas among 528 traced cases with cancer primary in the outer quadrants the survival rate was 64.6 percent. A review of these cases reveals that the various forms of treatment were rather equally distributed among those with inner and outer quadrant disease. Hence, location in the breast does not constitute a significant variable in the comparative analysis of treatment.

Stage I cancer therefore includes smaller groups in which the survival varies from approximately 40 or 50 to over 80 percent, but differences in most of these groups do not involve significant alteration in the forms of treatment.

In stage II Brooks and Daniel (*12*) differentiate those with isolated low-lying axillary nodes and Wassink (*13*) differentiates advanced disease on the basis of diffuse or fixed axillary-lymph-node involvement. In this series when the metastatic nodes are described as small, the survival rate averaged 28.6 percent; but when the nodes were described as large or extensive, the survival rate averaged 12.2 percent. Palliative measures were rather frequently used in the more advanced lesions, especially in the age group above 60 years.

In stage III, when the metastases were confined to the homolateral lymph nodes in the infraclavicular or supraclavicular regions, the prognosis was better than for any of the other so-called distant regions. Of 161 patients in this category, many of whom also had axillary lymphatic involvement, the survival rate was 5 percent; whereas among all others of the 414 white females in stage III of the disease only 2 persons, less than 1 percent, survived. Although radical surgery was seldom used for patients in stage III, when used it was principally in this more favorable group. Therefore, a more critical analysis of treatment in stage III would require the separation of those with homolateral lymphatic metastases in the clavicular region.

HISTOLOGIC CLASSIFICATION

The histologic classification of breast cancer varied greatly in different hospitals and reflected a lack of standardization of criteria and terminology among the various pathologists. One pathologist assigned modifying terms designating the type of cancer in over 99 percent of the patients biopsied in his hospital, while in another hospital less than 20 percent were diagnosed other than by the general term "cancer." Of the 15 most commonly used histologic terms only 1, adenocarcinoma, appeared in the reports of all hospitals, the frequency of this classification varying from 3.3 to 68.7 percent. One pathologist called the disease carcinoma simplex in 42 percent of his patients, while other pathologists never used this term. Some considered the presence of scirrhous elements as a critical differentiating point, while others felt that scirrhous elements merely represented the response of the tissues to the carcinoma and that their presence was not of fundamental importance. For these reasons, the histologic classification of breast cancer cannot be considered as a common variable in this study. Therefore, detailed reports of survival rates based on such classifications are not presented. In general, however, there was very little difference in the survival rates among the more common types, with 1 exception. Of 46 tumors diagnosed as malignant duct papilloma, practically all were early forms of can-

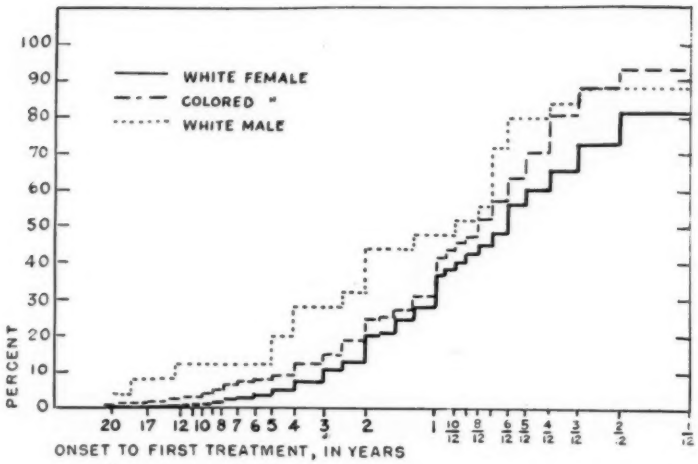

FIGURE 1.—Cumulative percent distribution of duration of disease prior to first treatment, based on history of onset, of patients with single primary breast cancer.

cer in stage I, and for this reason the survival rate was high.

Lest this discussion of the lack of agreement among pathologists in regard to terminology lead one to depreciate the importance of the pathologist in the control of breast cancer, it must be emphasized that the fundamental purpose of histologic study is to determine whether breast tumors are malignant or benign and to establish the presence or absence of early involvement of the axillary lymph nodes.

DURATION OF DISEASE

The duration of the disease at first treatment is shown in figure 1 for white females, Negro females, and white males. The mean duration for white females was 15.2, and for Negro females 22.6 months. Figure 1 shows that the cases may be divided into three rather equal groups: 4 months or less, 5 to 12 months, and 13 months or longer. In table 4 the status of these patients, based on their history of duration of the disease, is shown 5 years after first treatment. Although significant correlations existed between the duration of the disease and the stage of disease at first treatment, the duration of disease was not correlated with survival rates when corrections were made for the stage of the disease. Thus, patients having a history of short duration tended to have earlier stages of cancer than did those with long histories; but duration per se contributed little to the determination of prognosis which could not be more directly established by considering only the stage of disease at first treatment. At least two essential features contribute to this situation. Many patients are only vaguely familiar with the onset of their cancers; different cancers vary in their rate of growth, some grow rapidly and metastasize early while others grow slowly and may remain localized for years. If it were possible to determine accurately the onset and to correct for growth rate and for tendency to metastasize, the correlation between duration and survival would be much more striking than is here observed. The lack of such demonstration, however, does not detract from the obvious fact that for each cancer patient the earlier the treatment is begun the more favorable is the chance of cure.

TABLE 4.—*Status of white female patients with single primary breast cancer 5 years after first treatment, by duration of disease before first treatment*

Status of—		Duration of disease before first treatment							
Patient	Disease	Less than 5 months		5–12 months		13 months or longer		Unknown or unspecified	
		No.	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
Dead	Present	571	56.3	540	65.4	469	65.4	118	58.7
	Absent	45	4.4	36	4.4	22	3.1	5	2.5
	Unknown	4	.4	5	.6	3	.4	2	1.0
Total		620	61.1	581	70.3	494	68.9	125	62.2
Lost [1]	Present	19	1.9	27	3.3	32	4.5	14	7.0
	Absent	40	3.9	22	2.7	30	4.2	5	2.5
	Unknown	1	.1	1	.1	4	.6	1	.5
Total		60	5.9	50	6.1	66	9.2	20	10.0
Living	Present	74	7.3	51	6.2	46	6.4	22	10.9
	Absent	249	24.6	135	16.3	108	15.1	32	15.9
	Unknown	11	1.1	9	1.1	3	.4	2	1.0
Total		334	32.9	195	23.6	157	21.9	56	27.9
Grand total		1,014	100.0	826	100.0	717	100.0	201	100.0

[1] Lost signifies patients who have not been followed up as long as 5 years, and whose status 5 years after the initial treatment is therefore unknown.

CRITERIA OF DIAGNOSIS

Results of breast-cancer therapy are usually expressed for histologically proved cases. Under what circumstances is cancer diagnosed and treated without histologic reports? In this study, 21 percent of the patients diagnosed by the hospitals and accepted by us as having breast cancer did not have histologic reports available in their records. The status of these biopsied and unbiopsied patients 5 years after first treatment is shown in table 5. Considering the total follow-up rather than their status at 5 years, there was evidence of extension, metastases, or subsequent recurrence in all but 23 of the 591 white female patients in this group. The survival rates of these groups are shown in table 6. For each stage the survival rates were much less in the unbiopsied, the total survival rates averaging 14.2 percent in the unbiopsied and 32.9 percent in the biopsied. Most of these patients were treated with radiologic methods. It is clear that those breast cancers diagnosed without histologic reports were almost exclusively cancers in advanced stage. The inclusion of these patients greatly increased the proportion of cancer in advanced stage in the total series and furthermore tended to reduce the stage specific survival rates.

TABLE 5.—*Biopsied and unbiopsied white female patients with single primary breast cancer, all stages, 5 years after first treatment*

Status of—		No biopsy		Biopsy report	
Patient	Disease	*Number*	*Percent*	*Number*	*Percent*
Dead	Present	451	76.3	1,247	57.5
	Absent	9	1.5	99	4.6
	Unknown	0	.....	14	.6
Total		460	77.8	1,360	62.7
Lost [1]	Present	50	8.5	42	1.9
	Absent	4	.7	93	4.3
	Unknown	1	.2	6	.3
Total		55	9.3	141	6.5
Living	Present	35	5.9	158	7.3
	Absent	33	5.6	491	22.7
	Unknown	8	1.4	17	.8
Total		76	12.9	666	30.8
Grand total		591	100.0	2,167	100.0

[1] Lost signifies patients who have not been followed up as long as 5 years, and whose status 5 years after initial treatment is therefore unknown.

TABLE 6.—*Five-year survival rates of biopsied and unbiopsied white female patients with single primary breast cancer by stage of disease at first treatment*

Stage of disease	No biopsy report		Biopsy report	
	Patients traced	Survival	Patients traced	Survival
	Number	*Percent*	*Number*	*Percent*
I	49	18.9	550	60.3
II	120	12.5	929	23.9
III	186	1.1	210	4.3
IV	181	26.2	337	30.6
Total	536	14.2	2,026	32.9

CANCER THERAPY

The value of radical surgery in the treatment of breast cancer has been well-recognized in medical centers for many years. Since the report of Halsted (*14*) in 1894, numerous and critical evaluations have established radical mastectomy as an accepted method of treating carcinoma of the breast. On the other hand, Hartman (*15*), Fitzwilliams (*16*), and Grace (*17*) have advocated the use of simple mastectomy when evidence of axillary metastasis is lacking. With the development of and the improvements in methods of administering high-energy radiation, numerous writers have advocated the use of X-ray, preoperatively (*18–21*), postoperatively (*22*, *23*), and both preoperatively and postoperatively (*24–27*). Reports on the use of radium both interstitially and externally reflect differences of opinions in regard to its value (*9*, *28–31*).

In the present study, all methods of radical mastectomy are termed "radical surgery." Partial or complete mastectomy is classed as "local surgery." Radium is considered under 2 heads: external (pack, plaque, bomb, etc.); and interstitial (seeds, needles, capsules). X-ray is divided into 2 groups according to total dosage administered over the tumor-bearing region. When more than 1 of each of these forms of therapy are included in a course of therapy, they are listed in the order of their administration. The initial course of therapy is separated from all subsequent therapy given for prophylaxis, recurrence, extension, or metastasis, and each course of subsequent therapy is separated, except in stage III. Considering these 6 forms of therapy, their various combinations, order of administration, and the courses of therapy, it was observed that 505 combinations of treatment were used for the 2,945 patients with single primary breast cancer. Thus the treatment of cancer of the breast is highly individualistic and varied.

Although careful follow-up and subsequent therapy can do much to influence the course of the disease, the principal opportunity for cure rests on the eradication of the center at the initial therapy. All subsequent data herein are presented on the basis of the initial therapy without reference to the presence or type of subsequent therapy. Table 7 shows the distribution of surgical and nonsurgical forms of treatment of breast cancers, stages I and II.

TABLE 7.—*Initial therapy of breast cancer in white females by age groups*

Age of patient at onset (in years)	Cases grouped by treatment [1]				Percentage distribution			
	Radical surgery	Local surgery	Non-surgical	All others	Radical surgery	Local surgery	Non-surgical	All others
STAGE I								
Less than 40	51	32	2		60	37.6	2.4	
40 to 49	137	50	17		67.1	24.6	8.3	
50 to 59	107	45	20		62.2	26.2	11.6	
60 and over	77	61	55		39.9	31.6	28.5	
STAGE II								
Less than 40	120			43	73.6			26.4
40 to 49	250			67	78.9			21.1
50 to 59	239			80	74.9			25.1
60 and over	138			160	46.3			53.7

[1] In cases where the patient had both radical and local surgery, the treatment is classified under the former.

TABLE 8.—*Five-year survival rates of white female patients with single primary breast cancer, for common forms of initial therapy by stage of disease*

Stage	Group	Initial therapy	Total cases	Traced cases	
				Number	Survival
			Number		*Percent*
I	A	Radical surgery	152	132	68.9
		Radical surgery and postoperative X-ray (doses of 2,000 r or more)	70	60	70.0
		Radical surgery and postoperative X-ray (doses less than 2,000 r)	38	35	48.6
		Radical surgery and preoperative and postoperative X-ray (all doses)	43	39	69.2
		Radical surgery and preoperative X-ray (all doses)	23	22	54.5
	B	Local surgery	63	56	48.2
		Local surgery and postoperative X-ray (doses of 2,000 r or more)	52	46	60.9
		Local surgery and postoperative X-ray (doses less than 2,000 r)	28	25	40.0
	C	X-ray (all doses)	38	30	23.4
		Radium (all doses)	35	33	24.3
II	A	Radical surgery	300	283	28.6
		Radical surgery and postoperative X-ray (doses of 3,500 r or more)	59	54	35.2
		Radical surgery and postoperative X-ray (doses of 2,000–3,500 r)	89	89	29.2
		Radical surgery and postoperative X-ray (doses less than 2,000 r)	120	113	17.7
		Radical surgery and preoperative and postoperative X-ray (all doses)	94	77	18.2
		Radical surgery and preoperative X-ray (all doses)	42	40	20.0
		Radical surgery and preoperative or postoperative radium (all doses)	23	23	26.1
		Radical surgery and various combinations of both radium and X-ray	27	26	42.3
	B	X-ray (all doses)	115	95	5.3
		Radium (all doses)	50	49	14.3
III		X-ray in doses above 3,500 r	70	62	4.8
		X-ray in doses of 2,000–3,500 r	102	100	.0
		X-ray in doses less than 2,000 r	78	75	.0

The 5-year-survival rates for the more common forms of initial therapy are listed by stage of disease in table 8. The types and combinations of therapy, as listed, include 81 percent of the white female patients treated in stages I and II and 60 percent of those treated in stage III. The numerous combinations not listed were too small to warrant individual consideration. In table 8 for both stages I and II the treatment methods which include radical surgery are grouped separately from other methods. Although reports of negative examinations of axillary nodes were not routinely so complete as positive examinations, the examinations with radical surgery are roughly equivalent to pathologically proved cases in both stage I and stage II. These groups are, therefore, rather homogeneous both as to proof of axillary-disease status and as to one component of initial therapy. Groups not having radical surgery are principally of patients in which the presence or absence of axillary involvement was dependent on clinical judgment.

Although the histologic evidence of lack of lymphatic involvement will never be so complete with local surgical or with radiologic therapy as with radical mastectomy, the differences observed in the survival rates following local surgery (48.2 percent) and radical surgery (68.9 percent) are about what might be expected for these two procedures, one of which neglects the removal of those lymphatic chains and nodes so commonly involved.

The results obtained with the independent use of X-ray and radium do not approach, in terms of 5-year survival rates, the results attained with radical surgery. It is of more practical importance to evaluate the use of radium and X-ray in stages I and II by considering their use as an adjunct to surgery. Although too few data are available for stage I, for stage II the sur-

vival rates following the use of radium and radical surgery (26.1 percent) were about the same as those observed with radical surgery alone (28.6 percent); but the use of radium chiefly preoperatively and interstitially in the axilla, when combined with radical mastectomy and X-ray, was associated with good results in this small series.

X-ray was used sufficiently often with surgery in stages I and II to permit a consideration of dosage factors. Fully realizing the differences between tumor dosage and dosages measured on the surface of the skin or in air, we utilized the total skin dose per single course of therapy as measured in air to divide the patients into two main groups. The group receiving 2,000 r or more was in some instances subdivided into groups receiving more or less than 3,500 r. X-ray therapy given to the opposite side of the chest, to the ovarian region, and similar types of prophylactic treatment were not considered in calculating the dosage to the tumor-bearing region.

The groups given preoperative X-ray (table 8, stages I, A, and II, A) were too small to consider dosage factors, but in general these groups had relatively low survival rates as compared with other methods in these same general classifications. Groups with preoperative and postoperative X-ray were also too small to consider individual combination of dosage; but the combined doses of X-ray for all patients in these groups was over 2,000 r, and the results are similar to those obtained for patients treated postoperatively with over 2,000 r.

In groups I, A, II, A and B, and III those given less than 2,000 r of postoperative X-ray uniformly had lower survival rates than those receiving 2,000 r or more. Although in group I, A, little difference was observed, in groups I, B, and II, B, survival rates were somewhat better in those receiving high doses of postoperative X-ray than in those treated with the respective surgical procedures alone.

In stage I the group treated with radical surgery and postoperative X-ray in doses of 2,000 r or more had a relatively high survival during the first 2 years, but beyond this time the mortality was greater than in the group treated with radical surgery alone, so that by the end of the fifth year the survival rates were nearly identical. The group treated with postoperative X-ray in doses of less than 2,000 r had a relatively low survival rate. In stage II, the same trends were also apparent. The delay in mortality in the groups treated with high doses of postoperative X-ray as compared with the group treated with radical surgery alone can be expressed in the percentage of months lived, the maximum being 60 months for every patient in the group. The percentage of months lived for those in stage I was 88.2 and 83.5, respectively, and for those in stage II, 64.2 and 56.8, respectively.

The survival rates of patients with stage-III breast cancer in groups initially treated exclusively with X-ray were proportional to the dosage. The results, as measured at the end of the fifth year do not reflect the differences which were rather marked at the end of the first and second years.

Thus, when X-ray was given in high doses it was found to be associated with consistently better survival rates than when administered in small doses. The greatest interest here is in connection with the three groups in which surgery and surgery with varying doses of postoperative X-ray are compared. When the surgical treatment was supplemented with relatively high doses of X-ray, the survival rates were above those obtained with surgery alone, but when surgical treatment was supplemented with relatively small doses of X-ray, the survival rates were consistently lower than those obtained with surgery alone. The retesting of the homogeneity of the respective groups yielded no explanation in terms of the disease process or the selection of patients which would alter the results observed. It is possible that the extremely low survival rate seen in those with stage I cancer with low doses of postoperative X-ray could partly, but not completely, be explained by selection

factors, but these same considerations increased the relative difference observed in stage II.

The interpretation of these factors must be considered as tentative. When the survival data for those treated with radical surgery and those treated with postoperative X-ray in high doses were graphed, the curves tended to converge both in stage I and in stage II, and at no time were the differences on any one curve great enough to be considered as independently significant. For this reason, it appeared that in these two stages a 5-year period of follow-up is too short to permit adequate comparisons.

The consistently poor results observed following the use of small doses of X-ray deserve comment. A review of the literature reveals no evidence that survival rates following the use of small doses of X-ray have been evaluated under these circumstances, and no evidence was found that small doses of X-ray might actually be harmful in the initial treatment of human breast cancer. It is known, however, that small amounts of X-ray will facilitate the growth of transplanted tumors in animals (*32*, *33*).

Failures following surgical excision of breast cancer, regardless of the apparent stage of disease at the time of first treatment, obviously indicate that some viable cancer cells frequently remain in unexcised tissues. In the cases considered in the present study, it was apparent that the time required for these remaining foci of cancer cells to become manifest recurrences or metastases varied greatly; in one patient a recurrence was first recognized 18 years after treatment. Whether or not all of these cancer cells multiply to form recognized metastatic foci is unknown. On the evidence of 5-year survival rates, it might be assumed that the frequency with which such foci eventually grow and cause death is not greatly reduced by relatively large doses of X-ray, but the time involved seems to be prolonged. The frequency with which such foci become active is conceivably increased after the administration of small doses of X-ray. To what extent these results may be dependent on changes induced in the host or in the cancer cells is unknown.

Radical mastectomy was the preferred treatment administered in both stages I and II of breast cancer.

QUACKERY

The role played by quackery in the treatment of breast cancer deserves brief comment. Of 2,945 patients with single primary breast cancer, 72 had received various types of treatment by quacks prior to coming to the hospitals selected for this study, and an additional 18 interrupted their treatment to subject themselves to some form of quackery. About 2 percent of the white females and about twice as many colored females were included in this group. Not 1 cure was observed as a result of such treatment. The principle effect was the delay of proper treatment to such an extent that the survival rate in this group was less than half that of the total group of breast-cancer patients.

EXPERIMENTAL THERAPY

Although undesirable variation existed in the methods of applying high-energy radiation to breast cancer and in the forms of surgical therapy and although many of the younger patients were sterilized by ovariectomy or by X-ray, the use of new and truly experimental methods was confined to 25 patients. This number seems very low in view of the fact that more than 2,000 patients were eventually seen in an advanced incurable stage of breast cancer.

THERAPEUTIC METHODS

Without reducing the application of well-recognized methods of breast-cancer therapy and without neglecting individual considerations so necessary to proper medical care, it is suggested that the results attained in the treatment of early cancer might be improved by greater emphasis on fewer methods.

The occasional use of unusual methods of therapy in early cancer contributes little to our knowledge of cancer therapy unless their use is planned and correlated in numerous institutions. On the other hand, the results observed in advanced metastatic carcinoma of the breast should serve as a stimulus to the development and application of new methods of cancer therapy.

HOST FACTORS IN BREAST CANCER

Various general individual characteristics such as age, sex, color, and susceptibility to subsequent cancer must be considered in breast-cancer therapy. All these variables except age were held constant in the previous discussion by considering only white females with single primary breast cancer.

AGE

The percent distribution by age at first treatment is shown in table 9 by color and sex. The mean age of white females was 53.7 years, of Negro females 48.3, and of white males 62.2 The table shows that the largest single 10-year-age group for white females was 50 to 59 years. The total number of white females in this age group is compared with that of younger and also older groups with reference to their follow-up status 5 years after first treatment (table 10); the survival rate of the younger group was 31.4 percent; of the group 50 to 59 years old 26.1; and of the oldest group 27.4. These figures do not support the frequent contention that the younger cancer patients have a poor prognosis. The tendency of the younger patients to have a slightly better survival rate than older patients is associated in part with the fact that they tended to come to the hospital sooner than older patients and in part to the fact that the treatment of younger patients in stages I and II more frequently involved radical surgery. A review of the group below 40 years of age failed to reveal any significant differences from the group which included all below 50 years. In 10 of the younger patients the cancer was complicated by pregnancy. Only 1 of these patients entered in stage I, and the therapy was chiefly palliative. Although it is difficult to weigh properly all the various dependent variables involved, the age of the patient does not significantly influence the over-all results of breast-cancer therapy.

TABLE 9.—*Age at first treatment of persons with single primary breast cancer, by sex and color*

Age group	Female		Male	
	White	Colored	White	Colored
	Percent	*Percent*	*Percent*	*Percent*
10–19	0.03	.....	.....	.....
20–29	1.2	3.3	.....	.....
30–39	12.5	21.6	11.1	.....
40–49	26.7	34.0	3.7	.....
50–59	28.8	18.9	33.3	50.0
60–69	20.6	13.1	25.9	.....
70–79	8.6	1.3	14.8	50.0
80–89	1.5	2.6	11.1	.....
Unspecified	.07	5.2	.....	.....
Total	100.0	100.0	100.0	100.0

NEGRO FEMALES

The total number of Negro females and their distribution by stage of disease and by age are shown in tables 1, 2, and 9. Among 102 traced patients, 25 survived 5 years. The mean age of Negro females tended to be about 5 years younger than that of white females, the mean duration of the disease prior to first treatment about 7 months longer, the stage of disease advanced, and the follow-up poor. Despite the difficulties in estimating the result of therapy under these circumstances, the observed results were consistent with those for white females when correction was made for stage of disease at first treatment.

WHITE MALES

The 30 white males with breast cancer averaged 8½ years older than white females. Among 28 traced patients, 10 were living at the end of 5 years. This rather favorable survival was associated with the fact that relatively few were first treated in stage III of the disease. Except for the low incidence of breast cancer in males and the late age at onset, its behavior and response to treatment in males do not seem to be significantly different from its behavior and response in females in so far as can be determined in this study.

TABLE 10.—*Status of white female patients with single primary breast cancer 5 years after first treatment, by age at onset*

Status of—		Age at onset (in years)							
Patient	Disease	49 or less		50–59		60 or more		Unspecified	
		Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Dead	Present	729	60.8	479	65.0	418	59.6	72	59.5
	Absent	36	3.0	25	3.4	43	6.1	4	3.3
	Unknown	2	.2	5	.7	6	.9	1	.8
Total		767	64.0	509	69.1	467	66.6	77	63.6
Lost [1]	Present	34	2.8	23	3.1	27	3.9	8	6.6
	Absent	44	3.7	22	3.0	30	4.3	1	.8
	Unknown	2	.2	3	.4	1	.1	1	.8
Total		80	6.7	48	6.5	58	8.3	10	8.3
Living	Present	93	7.8	35	4.7	50	7.1	15	12.4
	Absent	246	20.5	136	18.5	124	17.7	18	14.9
	Unknown	13	1.1	9	1.2	2	.3	1	.8
Total		352	29.4	180	24.4	176	25.1	34	28.1
Grand total		1,199	100.0	737	100.0	701	100.0	121	100.0

[1] Lost signifies patients who have not been followed up as long as 5 years, and whose status 5 years after initial treatment is therefore unknown.

SECOND PRIMARY CANCER

A cross section of any older population group will reveal a rather high proportion of persons who at some time in the past have had cancer of the breast, skin, or lip, since the treatment of these forms of cancer is associated with high survival rates. In a selected group, the calculation of expected cancer incidence and distribution involves complicated correction factors for this back-tracking procedure. However, expected cancer-attack rates in groups selected at the beginning of an observation period can more readily be established.

If consideration is given to the white females who were first diagnosed and treated for breast cancer in the hospitals chosen for this study during the period selected by each hospital, the follow-up examinations from time of first treatment until death, and the loss from follow-up, or until the maximum of 5 years was reached, the number of new primary cancers recognized as developing in this period can be established. Using as a basis the age distribution of these white females and the time of follow-up for each, calculation can be made, on the basis of recently available incidence data (*34*), of the number of cancers that would be expected in the general population. Similarly, the deaths from new cancers can be observed and the expected values calculated by using the population and mortality data of the Bureau of the Census (*2*). The following tabulation shows the mortality and the incidence per 100,000 population, both expected and observed, of second cancers in a 5-year follow-up of 1,719 white females who had single primary breast cancer when selected initially:

	Number expected	Number observed
Death (cancers, all sites)	17.3	18.0
Cancer incidence:		
Breast	6.6	8.0
Other sites	22.4	22.0

The number of deaths observed and expected were very nearly the same, and this is true also of the total cancer incidence, 29.0 and 30.0, respectively. This evidence tends to exclude factors of immunity or increased susceptibility as playing significant associated roles during the 5 years of follow-up for this group.

APPLICATION TO CONTROL PROGRAMS

Before these findings are applied to cancer-control programs, it should be pointed out that the results attained in the treatment of any given stage of the disease were remarkably alike in the

different hospitals studied. Despite the difficulties involved in determining the optimal treatment for some patients, consistently good results were obtained with early cancer; the survival in advanced cancer, however, was uniformly poor. It should be pointed out further that with the present tendency to concentrate cancer care in treatment centers the results are probably applicable to the majority of breast-cancer patients in this country. In a recent survey by the United States Public Health Service (*34*) covering a population of 13,000,000, its 17,739 physicians, and 732 hospitals, 60 percent of the physicians had no cases, and another 25 percent had only 1 or 2 cases of any type of cancer during the year of the study. Reference of suspected cancer patients to a relatively few surgeons, radiologists, clinics, and hospitals, prepared for the diagnosis and treatment of cancer, is now common practice. Although gradual improvement in the application of known therapeutic methods can be expected to reduce fatality, such improvement will be limited as long as the first treatment continues to be initiated late in the course of cancer.

Cancer control involves cooperation between the potential patients, the physicians, the diagnostic and treatment clinics, the research centers, and the various professional, independent, and official public health agencies. It is not intended here to attempt an allocation of the functions and duties of each but rather to define a few of the crucial problems and where possible to suggest methods of approach. The 15,000 deaths from breast cancer each year (*2*) is sufficient reason why this form of cancer deserves direct consideration.

Most women now know the possible significance of a firm lump in the breast, and they are beginning to realize that proper and early surgical care is highly effective treatment. Despite the fact that relatively few women regularly examine their breasts and even fewer know how to conduct such an examination, approximately 90 percent of the lumps which eventually proved to be cancer were first observed by the patients. A breast cancer, not recognizable by a skilled physician, may in a few months grow to such an extent that the patient can readily determine its presence. Even if physicians routinely conducted careful and detailed physical examinations as often as once yearly, it is still probable that patients would continue to be first observers. If by haphazard and accidental methods such is the case, what can be expected if supervised, intelligent, and regular methods of examination were widely practiced? To get results the first observer must be an early observer.

It is obvious that the most hopeful approach to the recognition of early breast cancer is in the hands of each person, the immediate objective being to convert random, haphazard practices to planned measures of examination. It seems not unreasonable to suggest that in programs for the prevention of cancer every woman past 30 should learn from her doctor a method of inspecting and palpating her own breasts and that such examinations should be made not less than once each month. Some might object to this procedure on the grounds that it would create cancerphobia in many women. We do not believe that this is a valid objection. Such a condition would be induced only in extremely emotionally unstable women.

In both palpation and inspection, the position of the body is important. One method of facilitating bimanual palpation is to sit in a relaxed position with the spine, shoulders, and head bent forward. In this way the attachments of the breast are relaxed, and one hand may be placed below and completely behind the breast. Then, with the other hand in front, each small area of the breast can be felt between the finger tips, and corresponding parts of each breast can be carefully compared. A localized lump deserves professional opinion. Inspection of the breast directly and by the use of a mirror is also a part of the examination and for best results must be made with the person in different positions such as (1) sitting erect, (2)

with the arms above the head, and (3) lying on the back. If in one or more positions, a local dimpling of the skin or a retraction of the nipple is observed, or if a local bulge is apparent, a physician's interpretation of the findings is necessary.

Although the time lost by failure to discover early tumors cannot be measured, the delay after the tumor has been discovered is tangible, as shown in figure 1. Such losses can be measured not only in months but also in fatality. Once a tumor or suspected tumor has been found by the patient, immediate medical consultation is essential. Although the presence of normal variations, physiologic changes, and simple cysts can be determined by physicians through physical examination and transillumination of the breast, other pathologic conditions require more direct consideration. In some patients, the cancer will be obvious; in others it will be a possibility. Since these possible cases include the early forms of cancer, their management is crucial. Our studies have shown that repeated observations of suspected cancerous lesions are a form of procrastination either on the part of the patient, or the physician, or both, and as such contribute to the tragedy of late cancer. Early cancer can be excluded only with biopsy. It cannot be too strongly stated that in these cases biopsy is the only safe procedure. This involves immediate surgical exploration of the suspected lesion in the presence of the pathologist as consultant.

Once a diagnosis of cancer is made, there is usually but little delay in treatment; in the majority of cases treatment is instituted immediately. The chief obstacles at this point are the rejection of surgical procedures that have been demonstrated to be so effective in early breast cancer and the lack of available funds or facilities for treatment.

Successful programs for the control of breast cancer must facilitate early discovery, prompt medical consultation, biopsy, and radical treatment. Without underemphasizing the importance of research, trained cancer clinicians, and adequate diagnostic and treatment facilities, the reduction of mortality from breast cancer is most directly dependent on reducing the delay between onset and first treatment.

SUMMARY

The records are reviewed of 3,105 patients treated for breast cancer in 9 large hospitals. The status of these patients is presented in terms of the variables of treatment, of the cancer process, and of the host.

The terminology and criteria used in the histologic classification of breast cancer vary in different institutions and reflect local concepts rather than widely accepted principles.

Five-year survival rates following treatment of breast cancer are dependent on the regional extent of the disease at the time of initial therapy. When correction is made for stage of disease and type of therapy, other considerations such as age, sex, color, and duration of disease, show little correlation with survival rates.

Of the numerous therapeutic combinations, only the most commonly used forms of initial therapy are evaluated. When cancer is confined to the breast or to the breast and the axillary lymph nodes, the optimal treatment is radical mastectomy. X-ray therapy as a supplement to radical mastectomy is not associated with results significantly different from those obtained with radical mastectomy alone, but a tendency towards prolonged survival is seen following X-ray given in doses above 2,000 r, especially postoperatively when axillary involvement is confirmed.

Successful programs for the control of breast cancer must facilitate early discovery, prompt medical consultation, biopsy, and radical treatment. Emphasis is placed on replacing haphazard practices of first discovery by planned measures of early recovery.

It is suggested that every woman be taught a good method of inspecting and palpating her own breasts and that such examinations be made not less than once a month.

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CHEMICAL TREATMENT OF TUMORS. IX. REACTIONS OF MICE WITH PRIMARY SUBCUTANEOUS TUMORS TO INJECTION OF A HEMORRHAGE-PRODUCING BACTERIAL POLYSACCHARIDE[1]

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The isolation, from culture filtrates of *Escherichia coli* (*2*, *3*) and of *Serratia marcescens* (*4*), of the polysaccharide fraction which produces hemorrhage in mouse sarcomas, and the studies on the chemical nature (*5*) and physicochemical properties (*6*) of purified preparations of the active polysaccharide, were carried out with the aid of bioassay techniques (*7*, *8*) in which transplanted sarcomas were employed. Most of the published work, from this and other laboratories, dealing with the effect of bacterial preparations on tumors in experimental animals, likewise has been performed with transplanted sarcomas. Sufficient evidence, however, has been accumulated to show that the production of hemorrhage and necrosis by such agents is by no means restricted to transplanted sarcomas. While it has been found by most workers that, in general, carcinomas are more refractory than sarcomas, nevertheless several strains of transplantable carcinomas have been reported to be responsive to the action of these bacterial products. Some of these reports have been mentioned in an earlier communication (*4*).

Furthermore, this hemorrhagic and necrotic effect is not confined to transplanted tumors. Duran-Reynals (*9*) reported that a significant proportion of spontaneous mammary carcinomas in mice responded to the injection of washings and filtrates of bacterial cultures. In 1 experiment the carcinomas in 15 of 36 mice showed an intense reaction following a single injection. After repeated weekly injections, partial inhibition of tumor growth resulted in 8 instances, and 6 tumors regressed completely. Of these 14 cases of partial or total inhibition, 3 tumors had shown an intense hemorrhage reaction, 4 had given mild or doubtful reactions, and 7 had showed no reaction. Thus 26 of the 36 spontaneous carcinomas had responded to the injected bacterial material. Primary sarcomas induced in mice with methylcholanthrene-choleic acid (*10*) were found[2] to be sensitive to the hemorrhage-producing action of the active fraction of *Escherichia coli* culture filtrate. Andervont (*7*) injected culture filtrates of this organism into mice and obtained hemorrhage, followed by temporary regression, in a small percentage of spontaneous mammary carcinomas; hemorrhage was produced frequently with this procedure in primary sarcomas induced in mice with hydrocarbon carcinogens. Fogg (*11*), too, reported that bacterial preparations affected sarcomas induced in mice with a hydrocarbon carcinogen; he found that heat-killed bacterial cultures affected 4 of 9 induced sarcomas.

When a supply of the potent polysaccharide from *Serratia marcescens* culture filtrate became available (*4*, *5*), its effect on primary subcutaneous tumors was studied in experiments with 750 mice bearing tumors induced by injection of marginal doses of 3,4-benzpyrene.

MATERIALS AND METHODS

A and ABC mice of both sexes, obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, were placed on a diet consisting of unlimited amounts of Purina dog chow and tap water. Subcutaneous tumors were induced by the injection of a marginal dose (*12*) of 3,4-benzpyrene.

The animals were employed in the experiments when progressively grow-

[1] A preliminary report (*1*) was presented at the Chicago meeting of the American Association for Cancer Research, April 1941.

[2] Shear, M. J.: Unpublished work (1935).

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ing tumors appeared at the site of injection of the carcinogen. Since information was sought, among other points, on the effect of the size of the tumor upon the nature of the response to the bacterial product, tumors with diameters ranging from 3 to 20 mm. were used.

The hemorrhage-producing agent was obtained from culture filtrates of *Serratia marcescens* (*Bacillus prodigious*). Preparations of the potent polysaccharide fraction (*4*), containing about 1 percent of nondialyzable material, were kept in the refrigerator under toluene and were diluted as required. Bioassays with mice bearing transplants of sarcoma 37 had shown that these concentrates were highly potent: 0.1 cc. of a 1:10,000 dilution of these solutions was usually the end-point dose, i. e., the minimum dose required to produce detectable hemorrhage in about 50 percent of the tumors. This dose contained about 0.1 microgram of the polysaccharide. In the latter part of this work, trypsin-treated (*5*) polysaccharide became available; the results obtained with this material were similar to those given by preparations not subjected to digestion with trypsin.

For observations of induced hemorrhage, the tumors were cut open and inspected (*8*). Tumors in untreated control mice were pearly gray in appearance and were usually free of macroscopic hemorrhage. Occasionally, hemorrhagic tumors were encountered in the control mice, but almost invariably these were very large tumors. The small and medium-sized tumors in the untreated mice rarely exhibited hemorrhage.

Dr. H. L. Stewart's histologic examination of a great number of primary tumors, induced in strain A mice by the subcutaneous injection of hydrocarbon carcinogens in our previous studies in carcinogenesis, showed that almost all of these tumors were sarcomas. Microscopic examination of the tumors employed in this study was not carried out, but the tumors were considered to be sarcomas on the basis of their site and history of development, and on the basis of their gross appearance at autopsy.

RESULTS

TOXICITY

Exploratory experiments indicated that normal mice tolerated doses that were lethal to mice bearing induced subcutaneous tumors.

Single Injection

Preparation K, which had an end point of about 0.1 cc. of 1:10,000 dilution (*4*) and which therefore contained about 100,000 m. t. u. per cubic centimeter, was injected intraperitoneally into normal mice. Table 1 shows the doses, in terms of tumor units, and the number of deaths after 48 hours. Those that survived for 2 days usually recovered and appeared normal. From these data L. D. 50 for normal mice was about 1,000 m. t. u. Although no deaths were produced in normal mice with 200 m. t. u., this dose killed two-thirds of the mice bearing primary sarcomas.

TABLE 1.—*Survival of mice 48 hours after a single intraperitoneal injection of the hemorrhage-producing polysaccharide from* Serratia marcescens *culture filtrate*

Mice	Time	Number of animals surviving single dose [1]								
		10,000 m. t. u.	4,000 m. t. u.	2,000 m. t. u.	1,500 m. t. u.	1,000 m. t. u.	500 m. t. u.	200 m. t. u.	100 m. t. u.	50 m. t. u.
Control	Initial	8	8	23	36	26	8	8	.....	.....
	48 hours	0	0	3	13	13	7	8	.....	.....
With primary sarcomas	Initial	.....	.....	.....	.....	.....	.....	27	55	71
	48 hours	.....	.....	.....	.....	.....	.....	9	43	51

[1] The dosage, expressed in mouse tumor units, represents the number of minimum hemorrhage-producing doses injected.

TABLE 2.—*Survival of mice after repeated injection of large doses of the hemorrhage-producing polysaccharide from* Serratia marcescens *culture filtrate*

Experiment with—	Number of mice				Treatment				
	Initial	Surviving first injection	Surviving repeated injection for—		Number of injections	Period	Dosage in mouse tumor units		
			6 days [1]	12 days [1]			Initial	Final	Total
Normal mice:						*Days*			
a	149	40	36	33	2	9-16	1,000-2,000	1,500	2,500-3,500
b	4	4	4	1	4	7	400	10,000	16,400
c	8	8	8	8	9	10	200	16,000	18,400-30,600
Mice with primary sarcomas:									
d	27	9	8	4	9	12	200	400	1,300
e	18	14	6		3	4	100	10,000	12,100
f	9	8	8	8	7	12	100	2,000	4,800
g	17	11	10	9	7	14	100	2,000	12,100
h	22	22	20	19	9	10	50	2,000	13,350

[1] From the time of the second injection.

In the case of tumor-bearing mice, L. D. 50 was between 100 and 200 m. t. u. and was estimated to be approximately 150 m. t. u. Fatalities were obtained in tumor-bearing mice even with 50 m. t. u., but the deaths at low dose levels were usually confined to mice bearing large tumors; doses of this magnitude were better tolerated by mice with tumors which were small or medium-sized.

Repeated Injection

The results obtained in experiments with repeated injection of solutions of the polysaccharide are summarized in table 2. In most instances the agent was given by the intraperitoneal route alone; in some experiments, however, the injections were alternated between the intraperitoneal and the subcutaneous route.

Normal mice.—Of 149 mice which had been given a single large dose ranging between 1,000 and 2,000 m. t. u., only 40 survived. These results were in agreement with those given for this dose range in table 1. A second large dose of 1,500 m. t. u. was, however, tolerated by 33 of these 40 animals.

To ascertain whether the good survival following the second dose was a consequence of protection induced by the first injection or whether it reflected merely a selection of more resistant animals by the elimination of the susceptible ones with the first high dose, fresh mice were given small initial doses. From b, an exploratory experiment with only 4 mice, it appeared that increasing the dose level from 400 to 10,000 m. t. u. over 4 injections was too sharp a rate of increase, for 3 of the 4 mice died. It was found in the next experiment, c, that when a safe small dose of 200 m. t. u. was given first, and that when this was followed by daily injection of progressively larger doses, all of 8 mice tolerated 16,000 m. t. u. in a single final injection. Since it had previously been found (table 1) that an initial dose of 4,000 m. t. u. killed all of 8 mice and that L. D. 50 for an initial dose was 1,000 units, it appeared that the procedure employed in experiment c had afforded protection against a dose that was 16 times L. D. 50.

Mice with primary sarcomas.—Although an initial dose of 200 m. t. u. produced no deaths in normal mice, it killed two-thirds of the mice bearing primary sarcomas (table 2, experiment d). Repeated injection of relatively small doses (experiment d), with a maximum one of 400 units, resulted in the death of 5 of the 9 mice surviving the first injection. In experiment e, an initial dose of 100 m. t. u. killed only 4 of 18 mice with tumors, but rapid increase to 10,000 units in the next 2 doses resulted in the death of the 14 survivors of the first injection.

Better survival was obtained in 2 other experiments (f and g) in which the initial dose was likewise 100 units, but in which the dosage was increased gradually. In these 2 experiments most of the mice which survived the first injection also survived 6 subsequent doses which were gradually increased to 2,000 units.

The best results were obtained in experiment h. Here the initial dose was reduced to 50 units, with no mice killed by the first injection. Although 9 doses totaling 13,350 m. t. u. were given in 10 days, 19 of the 22 mice survived. It is to be noted that the final dose of 2,000 units, in experiments f, g, and h, was about 13 times L. D. 50 for tumor-bearing mice (table 1), and yet only a few deaths occurred in these 3 experiments, viz. 0, 2, and 3 of the 8, 11, and 22 mice, respectively, that were employed for the second and later injections. It thus appeared that tumor-bearing mice, like normal mice, developed a tolerance or resistance to a lethal dose following administration of small safe doses.

HEMORRHAGE AND NECROSIS

The hemorrhage and necrosis produced in primary tumors were found to be similar to those obtained in transplanted tumors. There have been few reports of histologic studies of the reactions of tumors to such agents. The most recent one is that of Gerber and Bernheim (*13*), who studied the changes produced in mouse sarcoma 180.

In control primary tumors with diameters of less than 15 mm., no hemorrhagic areas were evident and little if any necrosis was seen. However, when the untreated primary tumors attained a diameter of about 20 mm. or more, necrosis was more commonly seen macroscopically, and an occasional tumor exhibited areas of spontaneous hemorrhage. In the treated animals the area of the tumor exhibiting hemorrhage and the severity of the hemorrhage were usually proportional to the dose of polysaccharide injected, as in the case of transplanted tumors (*8*).

In rare instances the tumors appeared to be refractory to the action of the agent. On the other hand, a relatively small dose occasionally produced severe hemorrhage, sometimes accompanied by liquefaction, in the entire tumor; in these cases no unaffected tumor tissue was seen. When this occurred, however, the animal usually died within 24 hours after the injection. The production of severe hemorrhage throughout the tumor was not often seen in small or medium-sized tumors; it was usually obtained only in those tumors which were about 20 mm. or more in diameter.

EFFECT ON TUMOR GROWTH

In most instances, complete and permanent regression of the induced tumors did not occur following treatment with the polysaccharide. In some cases there was pronounced inhibition of tumor growth, and in a few instances there was complete and permanent regression. However, the tumors usually resumed growth and resulted in the death of the mice.

In animals killed at various stages of treatment, part of the tumor was seen to have been destroyed. Not infrequently most of the tumor was hemorrhagic and necrotic, but in these instances a portion of the tumor, particularly at the periphery, appeared unaffected. The surviving areas of the tumors continued to proliferate and to give rise to tumors of enormous size.

Areas of tumor refractory to an initial dose often appeared to be resistant to the action of subsequent doses of the polysaccharide. Repeated injection of this agent usually failed to destroy completely the remaining viable tumor tissue and, indeed, sometimes failed to produce hemorrhage in fresh areas. Many variations were employed in the dosage, route of administration, and in the spacing of the doses without achieving any noteworthy increase in the number of complete regressions. Subsequent administration of doses up to the sublethal level were apparently no more effective than smaller doses, and several injections daily no more effective than

injections at weekly intervals. The intraperitoneal, subcutaneous, intratumoral, and oral routes, alone and in combination, were tried. The oral and subcutaneous routes were usually ineffective in inducing hemorrhage. None of the techniques tried was found to supplement the effect given by the initial injection to the point where complete regression occurred. The possibility that frequent intravenous injections may yield better results remains to be tested.

The impression was gained that some sort of an immunity may have been developed following the first injection. This hypothesis would seem to be supported by the recent finding of Shwartzman (*14*) that this polysaccharide is an antigen in the rabbit.

OTHER EFFECTS

A single large dose produced symptoms resembling those of shock. The mice were prostrated, respiration was labored, diarrhea was not uncommon, muscle tone was decreased, and body temperature dropped. These symptoms developed within a few hours after the first injection and usually disappeared in 1 or 2 days, except in those instances in which all or most of the tumor was affected, in which event the animals usually died. Continued administration of large doses of this agent was often accompanied by loss in weight; this occurred in tumor-bearing and in normal mice, and in normal guinea pigs. These symptoms, however, were not observed in animals which received small doses, even though hemorrhage and necrosis were regularly induced. As with transplanted tumors, detectable hemorrhage was induced with doses as low as a fraction of a microgram.

Dr. Lippincott, of this Institute, examined histologically the tissues from a group of normal strain A mice which died within 24 hours after a single large dose. No significant lesions were found in any of the viscera in this preliminary experiment. These microscopic observations were in agreement with the findings reported by Shwartzman (*15*) and confirmed by later workers, that hemorrhage was not seen in normal tissues or organs following administration of potent bacterial preparations. Even when severe hemorrhage was produced in the tumors, none was evident in the normal tissues.

The changes in the tumors became visible within a few hours after the first injection, and severe hemorrhage was seen in about 5 hours. After repeated treatment, the tumors often were strikingly edematous; this condition was particularly noticeable in some of the tumors which did not show fresh hemorrhage after a series of injections. These gross observations are in agreement with the histologic finding of edema, reported by other workers, in transplanted tumors of mice treated with bacterial preparations.

ADSORPTION ON ALUMINUM HYDROXIDE

An adsorbent for the polysaccharide was sought which would, upon injection, serve as a depot for the steady release of the agent over a relatively long period. It had been found previously (*4*) that aluminum hydroxide removed the activity from aqueous solutions, but it was not known whether the precipitated aluminum hydroxide would release the polysaccharide after injection into mice.

Following the usage employed in the preparation of certain vaccines, a suspension of aluminum hydroxide was prepared by adding 0.5 cc. of a 10 percent sodium bicarbonate solution and 1.5 cc. of a 4 percent potassium alum solution to 10 cc. of water. The subcutaneous injection, into normal mice, of 1.0-cc. amounts of this suspension of aluminum hydroxide produced neither toxic symptoms nor ulceration at the site of injection. Thereupon this precipitate was formed in 10-cc. volumes of various dilutions of the polysaccharide. The aluminum hydroxide was centrifuged, resuspended in an equal volume of saline, and injected into mice with primary sarcomas. Hemorrhage and necrosis were produced with regularity in the tumors, showing that the polysaccharide was released from the adsorbent.

While repeated administration of such preparations caused severe hemorrhage and extensive necrosis in the tumors, and while the rate of growth of some of the tumors appeared to have been retarded, the tumors usually grew to a large size subsequently.

OTHER AGENTS

Since the techniques employed in administration of the polysaccharide did not result in complete regression of the tumors, except in isolated instances, exploratory attempts were made to enhance the action of this agent by concurrent administration of a variety of other substances. These were selected on the basis of one hypothesis or another; but since regressions were not obtained, neither the hypotheses nor the details of these experiments are given here.

Among the substances given, either alone or together with the polysaccharide, were: the sodium salts of maleic acid, fumaric acid, succinic acid, oxalacetic acid, and iodoacetic acid; cupric acetate; 1,3-dihydroxy-acetone; cystine; urea; methylene blue; and Aerosol O. T. (dioctyl ester of sodium sulfosuccinate). In some experiments, certain of these agents were given in combination with each other as well as with the polysaccharide. None of these substances, apart from the polysaccharide, produced hemorrhage in the tumors when safe doses were employed. Hemorrhage, sometimes extensive and severe, was, however, produced by some of these substances with lethal or sublethal doses. Sodium maleate, sodium oxalacetate, cupric acetate and sodium iodoacetate, and Aerosol produced hemorrhage in the tumors in a significant proportion of the mice, but only in amounts that were at or near the lethal. In these cases, the mechanism of the reaction appeared to be different from that involved in hemorrhage production by the polysaccharide, in that the latter was capable of regularly producing hemorrage in tumors with doses far below the lethal.

OTHER BACTERIAL SPECIES

The first injection of the bacterial polysaccharide resulted in the development of resistance, on the part of the tumor and of the host, to the action of subsequent doses. The nature of this resistance was unknown, but it was suspected that immunity might have been induced to this agent acting as an antigen. With this as a working hypothesis, attempts were made (January 1942) to circumvent the postulated immunity by employing a different potent preparation for each of the subsequent injections. Thus, the active polysaccharide of *Serratia marcescens* was given first; then, at intervals of several days, injections were given of culture filtrates of *Escherichia coli*, *Eberthella typhosa*, and of *Neisseria intracellularis*.

The varying of the bacterial material in this way yielded suggestive results in exploratory experiments. Some of the tumors were largely, or entirely, necrotic, and some of them showed severe and extensive fresh hemorrhage after subsequent doses. However, the mortality was high. The technique employed may have been too drastic, or the crude filtrates used may have been too toxic. It would be interesting to repeat and extend this line of inquiry with highly purified active preparations from various bacterial species after eliminating the toxic contaminants as had been done in the case of *Serratia marcescens*.

Zahl, Hutner, and Cooper (*16*) have also been studying the phenomenon of induced resistance to hemorrhage-producing agents. Their work is considered in some detail in the discussion.

DISCUSSION

While administration of this polysaccharide regularly produced hemorrhage and necrosis in these primary subcutaneous tumors, some portion of the tumor escaped destruction in most cases and continued to grow progressively until the death of the host. Both the tumor and the host developed re-

sistance to the action of subsequent injections, so that later doses were usually much less effective. Subsequent injections did at times produce hemorrhage and necrosis in some areas of the newly generated tumor tissue, but even when sublethal doses were given, the tumors usually continued to grow.

This experience and those obtained by other investigators with crude preparations of bacterial material might be taken to indicate that this line of approach is not worth pursuing further from the point of view of possibilities for therapy. However, the action of such agents is so selective for tumor tissue that it is considered worth while to attempt to follow this lead further.

A dose small enough to leave normal mice apparently unaffected produced hemorrhage and necrosis regularly in tumors without resulting in death or severe reactions on the part of the host. With some tumors, particularly the large ones, this same dose produced striking hemorrhage throughout the entire tumor, but in such instances the mice usually died within 1 or 2 days. Apart from tumors of unusual sensitivity, the degree of hemorrhage was commonly dependent upon the dose: the larger the dose, the more severe was the hemorrhage and the greater the proportion of affected tissue in the tumor. With doses that affected the greater part of the tumors, the mice usually died even though the same doses were tolerated by normal mice. (See tables 1 and 2.)

It thus appeared that the death of the tumor-bearing mice was largely ascribable not to the toxicity of the polysaccharide itself, but to some phenomenon resulting from the reaction of sensitive tumor tissue to the polysaccharide. The mice with tumors exhibited symptoms resembling those of shock, viz, prostration, labored respiration, muscular atony, lowered body temperature, etc.; in tumor-bearing patients this agent produced a fall in blood pressure (*17*). The working hypothesis which the author is employing at the present time is as follows: (1) Doses of polysaccharide, which are not lethal per se, damage the tumor; (2) the affected tumor produces changes which kill the host. Among the possibilities in (2) are: bleeding into the tumor with resulting decrease in blood volume; elaboration of toxic substances from damaged tissue; or a combination of the two.

From this point of view, measures which would prevent or counteract the secondary effects resulting from damage to the tumor might enable the animal to survive even though large amounts of tumor tissue were destroyed. Attempts to develop and apply measures of this sort, such as the employment of thiamine, of adrenal cortical extract, plasma, whole blood, etc., are in progress.

The reason for the primary effect of bacterial agents on certain types of tumors is still unknown. More than a decade ago, Gratia and Linz (*18*) considered the relationship of this phenomenon to anaphylaxis and immunity and concluded that this reaction was a type of hetero-allergy. Since then, other workers have contributed data and hypotheses along this line of thought. (See the section, Discussion, in an earlier paper (*4*).)

Barrett (*19*), in exploratory experiments, obtained hemorrhage in sarcoma 37 with horse serum in mice previously sensitized to this material whereas tumors in control mice, not previously sensitized, did not become hemorrhagic on treatment with horse serum. Of 60 sensitized animals, 55 exhibited shock, which was not seen in any of 59 nonsensitized control mice. Apparently these experiments dealt with a phenomenon different from that obtained with bacterial filtrates. In the first place, the latter type of agent produces hemorrhages in tumors without requiring a sensitizing dose. In the next place, the single injection of a bacterial preparation regularly induces hemorrhage in tumors in a dose range well below that which results in shock. As stated previously (p. 466), several substances were found capable of inducing hemorrhage

in tumors, but these differed from the specific bacterial agents in that the former class of substances had to be given in lethal or sublethal doses if positive results were to be obtained. There are apparently a variety of procedures which are so drastic for the host as to cause hemorrhage in the tumors. Severe shock sometimes appears to be capable of acting this way.

A distinction should be made, therefore, between the production of severe shock in the host resulting in hemorrhage in tumors, and the production of severe hemorrhage in tumors resulting in shock to the host. In both instances, hemorrhage and shock are obtained, but the sequence of events is reversed.

Duran-Reynals (*20*) investigated the possibility that reactive tumors might have been sensitized by the accidental presence of infecting organisms. He stated:

> In view of the fact that transplantable tumors are often infected by various bacteria, we have secured sterile tumors, and found that they are as sensitive to the phenomenon as the infected ones. Moreover, filtrates of tumors showing a strong phenomenon, injected into the skin of rabbits, have failed to sensitize this organ to intravenous injection of a potent bacterial filtrate made 24 hours later.

While many species of bacteria are capable of elaborating substances potent in inducing hemorrhage, many other species have given negative results. The specificity, moreover, goes beyond the species. In our first work in this field, we employed a colon bacillus (*2*, *3*). More recently, for experiments in which it was desired to circumvent induced resistance (see p. 466), potent filtrates of the colon bacillus were desired. Contrary to expectations, filtrates of the cultures of another strain of *Escherichia coli* were found to have negligible activity regardless of the culture medium employed, method of separation of the organisms, etc. On testing still other strains of *E. coli*, a second one was found to give filtrates of negligible potency whereas two other strains yielded filtrates of much greater activity. It thus appeared that different strains of the same species of bacteria may vary widely in potency for this phenomenon.

It would be interesting to isolate the active agent from a series of selected microorganisms and to make quantitative comparisons of potency in producing damage to tumors. The active agents, purified to the point where they were biuret negative, have been isolated from culture filtrates of two bacteria, viz, *Escherichia coli* (*2*, *3*) and *Serratia marcescens* (*4*, *5*). The former organism was employed because it had been used by Gratia and Linz in 1931 and by Shwartzman and Michailovsky in 1932; the latter organism was selected because it has been employed clinically in the form of "Coley's mixed toxins," i. e., "Erysipelas and Prodigiosus Toxins (Coley)" (*21*). There is no evidence for concluding that the metabolites elaborated by *E. coli* and *S. marcescens* have virtues which other potent organisms do not possess. In fact, it is likely that the active agent elaborated will be found to vary chemically, in certain respects, from organism to organism. Moreover, the potency in destroying tumor tissue, and the toxicity, may be found to vary widely from organism to organism when a series of these agents is isolated and when the biologic action of each is determined in terms of dry weight of purified preparations. Should this prove to be the case, purified preparations from other organisms might be found to have greater potentialities for attempts at therapy than those available at present.[3]

Moreover, attention in this regard should not necessarily be restricted to bacteria. Roskin and his collaborators,

[3] In a personal communication, Dr. J. B. Koepfli, of the California Institute of Technology, stated: "Dr. Seeley G. Mudd and I, together with our collaborators, have been investigating, since January 1941, the production, biological assay and chemistry of a mouse tumor regressant and hemorrhagic agent obtained from the culture of a strain of *B. coli* which we have had at the California Institute of Technology for several years. Thus far we have obtained about 10 grams of a purified material which, when injected intraperitoneally in a dose of 1–5 micrograms, consistently causes a moderate degree of hemorrhage with frequent complete regression in mouse Sarcoma 180. The tumors treated varied in diameter from 5–10 millimeters and had been retransplanted into brother and sister mated Marsh Buffalo mice. We have postponed further investigation for the duration of the present emergency."

in Moscow, have for a number of years been publishing provocative findings on the effect of *Schizotrypanum cruzi* on experimental tumors. The work along this line in Roskin's laboratory was summarized in a paper (*22*) published in 1939. In this connection, chapter VIII of Shwartzman's monograph (*23*) contains references to the literature on the effects produced on tumors by the spirochetes of relapsing fever and of tick fever, and by other organisms.

In the case of the Ehrlich mouse carcinoma and of the Flexner-Jobling rat carcinoma regressions were reported by Roskin following administration of the living trypanosomes, and of heat-killed preparations ("endotoxins"). Negative results were obtained with the other trypanosomes and other protozoa tested. A transplantable strain of rat sarcoma, however, gave negative results. It is noteworthy that no mention of induced hemorrhage was found in the original papers available here or in the abstracts of other papers. Roskin concluded that the therapeutic effect on the tumor was ascribable to a direct action of the toxin on the malignant cells.

While most of those who have made gross observations on the effects of bacterial preparations on tumors have stressed the violent hemorrhage produced and have given prominence to the action of these agents on the vascular system of the tumors, it is pointed out that those few who have studied the histologic changes have obtained the impression that the bacterial filtrates also had a direct action on the malignant cells (*7, 13, 24*). Sukhin (*25*), who injected cultures of *Bacillus prodigiosus* (*Serratia marcescens*) into rats bearing two strains of transplantable sarcoma and reported complete regressions in a significant number of the animals, also concluded that the bacterial factor had "a direct, highly destructive effect upon the cell of the tumour."

Harde and Kobozieff (*26*) and Boyland and Boyland (*27*) found a marked decrease in the ascorbic acid content of transplanted tumors in which hemorrhage had been produced by bacterial filtrate. This led Andervont and Shimkin (*28*) to administer ascorbic acid together with culture filtrates of *Bacillus prodigiosus* (*Serratia marcescens*). They reported that ascorbic acid prevented the appearance of hemorrhage in, and the resultant regression of the tumors, and postulated that—

> bacterial filtrate, by lowering suddenly the ascorbic acid content of the tumor, weakens its fragile capillaries, with resultant hemorrhagic extravasation.

This inhibition by ascorbic acid was not, however, duplicated in our hands when the purified active polysaccharide from the same strain of the same organism was employed (*5*).

In the hands of Shimkin and Zon (*29*), 0.002 mg. of one of our preparations of this active polysaccharide produced hemorrhage in over 90 percent of the tumors (sarcoma 37) within 6 hours; no significant drop in the red-blood-cell count was produced, but the platelet count within this 6-hour period dropped from a normal value of 500,000–900,000 to as low as 200,000. Gross and histologic examination failed to reveal hemorrhages in the kidneys, intestines, or lungs. These workers injected antiplatelet serum intravenously into mice bearing sarcoma 37. A dose of this serum which caused the platelet count to drop within 4 hours to less than 200,000, the level produced by the bacterial preparation, did not induce hemorrhage in the tumors.

With a larger dose of antiplatelet serum, the platelet count dropped to below 50,000, and most of the animals died within 24 hours. This dose produced petechial hemorrhages on the toes, the clipped ears, and in the intestines and lungs. With the higher doses of the antiplatelet serum, hemorrhage appeared in the tumors as well, but the extent of the hemorrhage did not approach that obtained with the bacterial agent.

The effect of snake venom was also compared, but here, too, hemorrhage in the tumors was not induced except with

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sublethal doses. These authors stated that, since simple reduction of the platelets does not produce hemorrhage in the tumors, the active agent in bacterial filtrate is probably a factor toxic for the capillaries and that the drop in platelets is apparently incidental, and is evidently not the primary cause of the hemorrhage produced in tumors.

It is obvious that much has yet to be learned concerning the mechanism by which such bacterial agents damage tumors and the phenomena which cause death when tumors are severely affected. Ten years ago Duran-Reynals (*20*) stated:

> The mechanism of death as a result of the reaction in the tumor is not understood, and no significant general lesions have been found at the autopsies.

Shwartzman (*23*) was of the opinion that—

> the apparent susceptibility of mice to the lethal effect of the active principles may be due to a sensitization following secondary bacterial infections of the tumors.

As regards the effect produced on the tumor, Shwartzman (*23*) stated in 1937:

> The mode of elicitation of regression and the nature of the reaction obtained remains, however, unknown.

Little light has been thrown on these problems since the foregoing statements were written.

The findings with primary induced tumors in mice are in many respects in agreement with those obtained by workers (*7*, *20*, *23*, *30*, *31*) who employed other types of tumors. For one thing, the lethal dose for animals bearing susceptible tumors is lower than for normal control animals. For another, larger tumors usually react more vigorously than small ones. Moreover, although many tumors are profoundly affected, complete and permanent regression of the tumors is not obtained regularly.

Zahl, Hutner, and Cooper, and their collaborators (*16*, *31–35*) stressed the occurrence of tumor-hemorrhage potency in gram-negative bacteria and its absence in gram-positive organisms. Some exceptions were noted with both types of organisms, but their work (*35*)—

> led to the postulation of the existence of a common toxic factor as the incitant of tumor hemorrhage among substantially all gram-negative bacteria.

They (*31*) inferred that the tumor-hemorrhagic agent was probably identical with a component of the complete endotoxin antigen, and stated (*16*):

> The preceding papers of this series suggest a uniformity in constitution and in toxic action of the complex O antigens of nearly all gram-negative bacteria. The widespread occurrence among gram-negative bacteria of the tumor-hemorrhage incitant and its correlation with the occurrence of O antigens was adduced as evidence for the existence of a single uniform toxin in the O antigens of gram-negative bacteria.

These authors have reported interesting results on several aspects of the action of bacterial preparations on tumors and on the host mice. Where the experiments were of a similar nature in their laboratory and in this one, the results have been in general agreement. Our observation (*1*) that both normal and tumor-bearing mice develop a tolerance for lethal doses following the administration of small doses of the active fraction from *Serratia marcescens* has been extended by similar findings of Zahl and Hutner (*33*) with potent preparations from *Salmonella typhimurium*. In quantitative bioassays (*8*, *36*) for hemorrhage-producing potency, we had found that transplanted tumors accidentally contaminated with bacteria were much more resistant to the agent administered than were sterile tumors. This finding agreed with that of Gardner, Bailey, and Hyde (*30*) that when their tumors ulcerated, there was a definite decrease in reactivity to hemorrhage production. They stated:

> Complete bacterial sterility of the tumors is an absolute necessity, if an end point in titration of active bacterial substances is to be attained.

With primary induced tumors we had found that tumor tissue which escaped destruction after the first injection of the active agent appeared to have a greatly increased resistance to the ac-

tion of subsequent doses. Light has been thrown on these observations by the recent experiments of Zahl, Hutner, and Cooper (*16*, *35*).

In one set of experiments they (*16*) immunized mice by intraperitoneal injection, over a period of 6 days, of sublethal doses of an active concentrate from cultures of *Shigella paradysenteriae*. Ten to fifteen days after the first injection, sarcoma 180 was implanted in these and in untreated control mice. When the tumor implants were 7 days old, the mice were given intraperitoneally an injection of the same preparation as had been used for the immunization. Tumors in the immunized mice were found to be resistant to doses of this agent which regularly produced hemorrhage in the tumors of the control mice. In addition, L. D. 50 for the immunized mice was four times as great as for the controls. In a later paper they (*35*) employed active concentrates from two closely related and from one taxonomically remote bacterial species in similar immunization experiments and concluded:

> Protection by immunization was found to be about as effective against the induction of tumor hemorrhage when the heterologous organisms were used as when the homologous were used.

These authors have contributed new data of various types, and several hypotheses. Among the latter is their inference that the hemorrhage-producing agent is an endotoxin. While this may prove to be the case there is as yet, to our knowledge, no published data which establish this point. In the same category would appear to be their postulated identification of the hemorrhage-producing agent with a component of the O antigens. They prepared potent concentrates of various organisms, but the chemical data obtained were insufficient to permit characterization of the active agent or deductions regarding the purity of the concentrates.

At the present time there are so few leads which offer promise for chemotherapy that any agent which has a selective effect on tumors is worthy of further study even though it destroys only part of the tumor tissue. While the work in this laboratory with bacterial substances is an outgrowth of the work of Gratia and Linz (*18*) on a transplantable guinea pig sarcoma treated with culture filtrates of *Escherichia coli* there are clinical observations, of which some were mentioned in a previous paper (*37*, *pp. 83–84*) in this series, which appear to parallel those on experimental tumors.

The work of W. B. Coley, who employed clinically "the mixed toxins of erysipelas and Bacillus prodigiosus," is widely known. Coley's first publication (*38*) on this subject was in 1891 and his last one (*39*) in 1936. His lifelong interest in the effect of bacterial preparations on human tumors was aroused by a case in which a sarcoma had recurred four times after operation and which disappeared after the patient spontaneously contracted erysipelas; the man was still well 7 years later. Ten years before Coley's paper appeared, Fehleisen had isolated a streptococcus from erysipelas lesions; in 1882 he (*40*) reported that this organism, administered to a woman with multiple sarcomas of the skin, elicited a typical attack of erysipelas. Moreover, the main tumor mass receded, and some of the smaller tumors regressed completely.

The clinical literature contains a considerable number of cases in which a favorable influence upon the course of the disease was reported following a spontaneous attack of erysipelas, or other severe infection, or following the experimental infection of cancer patients with erysipelas. The earliest experiment of this type was carried out by Professor Busch, of Bonn, in 1867. He (*41*) had previously reported in detail two cases of sarcoma in which the tumors had shrunk markedly after a spontaneous attack of erysipelas. Busch then attempted to induce erysipelas in a woman with multiple sarcomas. His first attempts to transmit the infection failed, but he persisted and finally succeeded in provoking an attack of erysipelas. From the historical point of view,

Busch's work is of especial interest since it was carried out before the development of modern bacteriology. Following the appearance of the erysipelas lesions in this patient, the large tumor shrank about 1 cm. per day and some of the smaller tumors were completely resorbed. However, the large tumor subsequently grew rapidly again.

While reports of complete and permanent regressions of human tumors following such infections are rare, partial and temporary regressions continue to be reported. In 1933, for example, Rosenrauch (*42*) described a case of sarcoma with metastases, histologically verified, in which an attack of erysipelas intervened; almost all the metastases disappeared, while the primary tumor shrank considerably. However, the effect was only temporary, and the tumors grew again. In 1939 Sakharov and Rossiisky (*43*) summarized the results of treating 113 patients with advanced cancer; some of the patients were given, in addition to an aqueous extract of adrenal cortex, large doses of gonococcal vaccine. In 9 percent of the cases arrest of growth, some regression, and clinical improvement of symptoms were recorded, but here, too, the favorable effects were only temporary.

Lilienthal (*44*) has recently summarized two cases which had been successfully treated with Coley's toxins. One patient, with a tumor diagnosed histologically as a pigmented sarcoma, had a recurrence after operation and was given Coley's mixed toxins. Lilienthal reported that—

> there was general amelioration to an astonishing degree, with rapid decrease in the size of the tumor. * * * He remained well for about nine years when he died of a disease not associated with new growth.

The second patient had a mediastinal tumor extending into the spine, which was found to be inoperable. Three pathologists (Mandelbaum, Gross, and Ewing) examined the sections; all three diagnosed the growth as a malignant tumor, although each classified it differently (angiosarcoma, hemangioepithelioma, and malignant cellular tumor of embryo type, respectively). The 2-year-old girl was treated with Coley's toxins and was still alive 19 years later. Lilienthal stated that in 1943:

> I found her *well* and exceptionally active at the age of 21. The thoracic scar was in the soft parts of the upper right thorax only.

The literature on the effect of certain infections and of bacterial preparations upon clinical cancer was reviewed by Loeb (*45*) in 1910, and "Erysipelas and Prodigiosus Toxins (Coley)" was included in New and Nonofficial Remedies by the Council on Pharmacy and Chemistry of the American Medical Association (*46*). The situation was reviewed again in 1934, and this preparation was retained by the council (*21*)—

> with a view to facilitating further studies with the product, especially in connection with its use as a prophylactic in conjunction with conservative or radical surgery. Its use in definitely inoperable cases may be quite justified, in many instances, as a desperate attempt to combat the inevitable.

In referring to Coley's work, a recent report of the Memorial Hospital stated (*47, p. 37*):

> His method of treating sarcoma with the toxins of erysipelas and bacillus prodigiosus, begun in 1899, is the only constitutional remedy which has withstood the test of time.

While clinicians have reported an occasional good result following the use of bacterial preparations, it has been the usual experience that such agents have been without beneficial effect upon the progress of the malignant disease in most instances. If attempts are to be made to obtain good results in a higher percentage of cases, several points should be considered. In the first place, past experience indicates that sarcomas are more likely to be responsive than carcinomas, and that selection of suitable cases of sarcoma would be preferable to random choice of any case of malignant disease. Secondly, unless preparations have been assayed for their effect upon animal tumors, the patient may be given material which is weak, or even entirely lacking in potency. Furthermore, proper control of

dosage is impossible unless the potencies of the various preparations used are known in quantitative terms. The need for some quantitative criterion of potency was felt as early as 1907 by Beebe and Tracy (*48*) who, in order to have some basis for comparison of different preparations, arbitrarily employed the nitrogen content. They recognized that there was no theoretical justification for their selection, inasmuch as a correlation had not been established between nitrogen content and potency, but they employed the nitrogen value as a criterion for lack of a more valid one.[4] Finally, some of the undesirable reactions of patients who have received crude preparations may perhaps be avoided if the preparations used are freed from toxic contaminants. Methods of purification of the active agent and of assay for potency are now available (*4, 5, 8*), at least as far as the agent which causes hemorrhage and necrosis is concerned.

Even when the foregoing considerations are borne in mind, it is unlikely that satisfactory results will be obtained in a high proportion of instances unless more information is obtained on the mechanisms by which such agents affect tumors and on the mechanisms which, apparently secondarily, produce prostration after a tumor reacts vigorously to the treatment.

Symptoms nowadays considered as indicating the occurrence of shock were noted as early as 1868 by Busch (*41*), who described a drop in temperature to below 30°, a weak and intermittent pulse, and pallor which developed, in a patient with regressing sarcomas, 2 weeks after he had succeeded in provoking erysipelas. He considered the untoward symptoms as consequences of resorption into the circulation of decomposition products of destroyed tumor tissue, a view arrived at more than 70 years later by Brues and Shear (*17*) on the basis of the high uric acid content of the blood and urine of patients treated with a potent preparation from *Serratia marcescens*.

Shock was mentioned by Gardner, Bailey, and Hyde (*30*), not as a consequence of damage to the tumor, but as probably being responsible for the hemorrhage They stated that in all probability the hemorrhagic phenomenon is a result of generalized vascular shock. Zahl and coworkers (*31*) mentioned the enhanced susceptibility to shock of treated animals bearing tumors as compared with normal nontumor-bearing animals. The observations on shock in mice bearing primary sarcomas after treatment with our polysaccharide have already been discussed (pp. 465, 467).

In the early clinical literature on the reactions of cancer patients subsequent to a spontaneous or induced attack of erysipelas, "collapse" was noted in patients in whom the tumor was regressing. In 1888 Bruns (*49*), professor of surgery at Tübingen, reported a case of melanotic sarcoma which recurred after operation; Professor Schüppel made the microscopic diagnosis of melanosarcoma. The patient contracted erysipelas, the melanotic growth began to recede, and regressed completely. Subsequent examinations by Bruns revealed that the patient remained well up to the time of the report 7 years later. Bruns gave a critical review of the literature to 1888, with 27 references. The case reports, including the histologic findings when given, were summarized and evaluated. In many instances the tumors receded temporarily but resumed growth. In a few cases, however, the regression was complete, with no recurrence over a period of many years. Twenty-two cases were reviewed, of which 5 were sarcomas in which the diagnoses were confirmed histologically. Of these 5 sarcoma cases, "sind 3 Fälle volkommen und dauernd geheilt."

Since these early observations, enough evidence has accumulated to show that neither an attack of erysipelas nor administration of bacterial "toxins" can

[4] From the historical point of view, it is worthy of note that, as early as 1907, Beebe and Tracy (*48*) administered cultures of *Bacillus prodigiosus* to animals bearing transplants of "a spontaneous tumor," and recorded the changes produced in the tumors. They found it necessary to increase the size of subsequent doses "as some tolerance had been acquired and the smaller doses failed to produce any reaction."

be expected to destroy tumors except, perhaps, in occasional isolated instances. However, the fact that such isolated instances have been reported raises the question whether these cases may not constitute a lead susceptible of development. In the words which Coley (*38*) employed in 1891—

> Nature often gives us hints to her profoundest secrets, and it is possible that she has given us a hint which, if we will but follow, may lead us on to the solution of this difficult problem.

As far as experimental tumors are concerned, the obstacles in the way of obtaining complete regressions with regularity are numerous. Usually the first injection of polysaccharide does not affect the entire tumor. The reason why some areas are resistant is unknown. Subsequent doses are usually less effective than the first one; both the tumor and the host develop increased tolerance to this agent. This resistance may also be conferred by substances from bacterial species unrelated to the one employed as the hemorrhage-producing agent. Whether the induced refractoriness is immunologic in nature or whether some other phenomenon is involved is still not certain. Perhaps it may be found possible to prevent the development of this tolerance, or to break it down. In that case, measures must be sought to prevent death from shock, for when large amounts of tumor tissue are suddenly destroyed, the animal usually dies in collapse.

These are but a few of the problems encountered. More information about the various mechanisms involved is required before it can be even surmised whether this line of approach is capable of being developed to the point of useful application.

SUMMARY

A polysaccharide fraction from culture filtrates of *Serratia marcescens* was administered to 750 mice bearing primary subcutaneous tumors induced by injection of a marginal dose of 3, 4-benzpyrene. This bacterial material regularly produced hemorrhage within a few hours in the primary tumors in doses as low as a fraction of a microgram. The results obtained with primary tumors, following administration of this agent, closely paralleled those obtained earlier with transplanted sarcomas.

For mice bearing primary subcutaneous tumors L. D. 50 was found to be about 150 m. t. u. whereas L. D. 50 for control mice was 1,000 units. For the preparations employed, these doses corresponded to 15 and 100 micrograms, respectively, of dry material.

When normal mice were first given a small dose, and when the amount administered was increased progressively, the animals developed a tolerance and survived a single injection that was 16 times L. D. 50. When mice with primary tumors were treated in an analogous fashion, they developed resistance to the lethal effect of an injection that was 13 times L. D. 50 for tumor-bearing mice.

Doses that were readily tolerated by mice with small or medium-sized tumors often were fatal to animals bearing large tumors.

With tumors of the same size, the degree of hemorrhage produced was dependent upon the dose: the larger the dose, the more severe was the hemorrhage and the greater the proportion of affected tissue in the tumor. With a given dose, the larger the tumor the more severe and extensive was the hemorrhage.

When small doses produced a striking degree of hemorrhage throughout the entire tumor, the mice usually died within 1 or 2 days. Death produced in this way appeared to be ascribable not to the toxicity of the polysaccharide itself, but rather to some phenomenon resulting from the reaction of a large amount of sensitive tumor tissue.

Areas of tumor unaffected by the initial dose were often resistant to the action of subsequent doses. Tumor tissue newly generated during a course of treatment was commonly refractory. Thus, in addition to the development of a tolerance on the part of the host to the lethal action of this agent, the tumor

tissue appeared to develop a resistance to the hemorrhage-producing action of the polysaccharide.

The active agent was adsorbed by aluminum hydroxide and was released from the adsorbent upon injection into mice.

While administration of this polysaccharide regularly produced hemorrhage and necrosis in these primary tumors, some portion of the neoplasm usually escaped destruction and continued to grow progressively until the death of the host. In the few instances in which the entire tumor appeared to have been severely affected, the animals developed symptoms of severe shock and died.

The relevant clinical literature is discussed in the light of these findings.

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EXPERIMENTAL ROENTGEN INJURY. I. EFFECTS ON THE TISSUES AND BLOOD OF C3H MICE PRODUCED WITH SINGLE SMALL WHOLE-BODY EXPOSURES

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INTRODUCTION

The effects of high-energy radiation upon normal and neoplastic tissues have been described by numerous investigators.[2] The terminal phases of tissue changes resulting from exposure to such radiations are usually described as tissue atrophy, parenchymatous cell loss, connective tissue overgrowth, and neoplasia. Further, it is generally accepted that the physical factors which contribute to the variety of end results are (1) the amount of radiant energy absorbed in the tissues, (2) the distribution of such energy within the tissues, and (3) the manner in which the exposures are made.

Although the cell as a unit may be injured or destroyed by radiation, it is necessary to go beyond cell changes and deal with modifications of inter-tissue and inter-organ relationships if any comprehensive understanding is to be gained regarding the various end effects. The chain of cellular and tissue changes which are initiated by exposure to radiation and which lead to terminal effects are complex and only partially understood.

Numerous accounts have been given of irradiation changes in various organs at some given time after treatment or exposure, but as yet little has been done relating the various changes as they take place progressively. In the studies reported in this series, correlations have been made of certain blood and tissue changes on a time basis. The chief aspects considered were (1) the kinds of tissue injury which followed irradiation, (2) the optimal irradiation conditions (dose, method of exposure, etc.), (3) the functional relationship of cell types under various conditions of injury and repair, (4) possible subliminal changes and tissue reserves, and (5) the histologic background out of which malfunction (including neoplasia) may arise.

Hematopoietic tissues have proved especially useful in such considerations because they show important changes both of morphologic and functional character, and because a continuous record of functional behavior can be obtained through a study of peripheral blood. By correlating the histologic conditions with blood picture at different times, it was possible to obtain information regarding the changes that precede and possibly determine some of the various terminal effects in the animals. Two strains of mice, one strain of guinea pigs, and one strain of rabbits were used. In some cases the different types of animals were employed in order to gain certain experimental advantages, and in others simply because they were more readily available.

Although much has been written regarding blood and tissue changes produced by ionizing radiations, most published descriptions have dealt only with changes of considerable magnitude. Since in occupational and inadvertent exposure of human beings to radiation long intervals usually occur between exposure and the appearance of injury, and inasmuch as the exposures often consist of a series of applications (any one or several of which would probably produce no perceptible effect), attention is drawn to the minimal or threshold changes. Hence it is desirable to know the nature of the smallest perceptible changes and the time of occurrence and duration of such changes. In this paper

[1] With the technical assistance of Jean W. Thompson, hematology, J. F. Marshall, histologic preparations, and H. L. Meyer, technical management of the experiments.

[2] For recent reviews, see Dunlap (*1*) and Downey (*2*). Also of interest are the papers of Heineke (*3, 4*), Cramer (*5, 6*), Desjardins (*7*), and Goodfellow (*8*).

are described the alterations produced by a single acute dose of radiation barely large enough to produce detectable changes. Later papers will deal with effects produced by different-sized doses delivered in various ways and also with the changes as seen in different kinds of animals. The first studies, therefore, serve as foundation experiments for the investigation of subthreshold changes and their possible accumulative effects.

MATERIALS AND METHODS

C3H mice from an inbred strain maintained at the National Cancer Institute were used exclusively in the first study. The animals were 6 to 10 weeks old when treatments were given. From certain preliminary experiments, it was known that doses of 50 r applied to the whole body of such animals produced faint but nevertheless detectable blood and tissue changes. Accordingly, doses of 50 r were used throughout.

Small-dose-irradiation effects are difficult to distinguish from changes produced by other agents or changes which sometimes appear to arise spontaneously. Hence, in establishing the effects attributable to radiation, three criteria were used: (1) A consistent difference between control and irradiated material; (2) a proper time relation between the responses of control and irradiated material; and (3) an orderly progression in the amount of injury following different-sized doses of radiation.

The X-ray treatment conditions used were as follows: 200 kv., 20 ma., 0.5 mm. copper and 1.06 mm. aluminum filter, and 105.3 cm. distance. The radiation intensity, as measured in air with a Victoreen ionization instrument, was 8.0 r per minute. Actual exposures were accomplished by placing the animals (10 per treatment) in a shallow celluloid tray with cover, which in turn was placed in the central part of the X-ray beam at the distance specified.

The celluloid sheets comprising the floor of the tray and the cover were each 1.5 mm. in thickness. An ionization chamber placed within the tray showed the intensity to be increased 1.5 percent when compared with the measurements made in free air. By placing masonite strips in the tray on either side of the chamber and also above and below in such a manner as to simulate a condition with the chamber at the middle of the mice during treatment, the intensity was decreased 1.5 percent. Thus the decrease in intensity due to absorption was compensated for almost exactly by scattered radiation. Accordingly, in this report and also in the other reports of this series, doses are based on measurements made in air, with the assumption that "air doses" and the "tissue doses" for the conditions used were very nearly the same (where mice were used). Frequent checks were made with the ionization chamber on the output of the X-ray equipment. The extremes of variation in intensity did not vary more than 5 percent throughout the whole course of the work, and no corrections were made by changing the exposure intervals.

The X-ray equipment was provided with a set of automatic time switches which increased the voltage according to a regular time pattern. It was also provided with an electric timer connected with the second automatic switch. Thus, in operation there was an interval after the voltage was first applied before the timer started, and likewise an interval after it started before full voltage was reached. By regulating the time intervals of the starting cycle, it was possible to obtain enough radiation (as measured by the Victoreen instrument) before the timer started to compensate exactly for the loss of dosage owing to incomplete voltage during the second part of the cycle. The complete cycle as finally adjusted required about 11 seconds, so that five starts could be completed in 1 minute. Tests carried out to determine the accuracy of the timing showed that a continuous exposure of 1 minute gave the same Victoreen reading (within 0.1 percent) as did an

exposure of 1 minute which included five starting cycles.[3]

Thirty-nine animals were used for the present experiment. Blood counts were made and tissues fixed at the following times after irradiation: 1, 2, 4, 8, 12, and 24 hours, and 2, 4, 7, 10, and 14 days. The blood studies consisted of total leukocyte and differential counts, coverslip smears and Wright's stain being used. During the experiment, three animals were examined each time a count was made and the same three animals were killed for tissues. Three control animals were killed at the beginning, and three other controls at the end of the experiment. Zenker-formol fixation (over night) and hematoxylin-and-eosin stains were used throughout. Routine paraffin embedding was done, and sections were cut at 10μ. For the most part, the following tissues were sectioned; testis, bone marrow (from the sternum and vertebral bodies), lymph nodes, spleen, thymus, spinal cord, intestine, stomach, lungs, kidneys, liver, and skin.

HEMATOLOGIC FINDINGS

Figure 1 shows changes in leukocyte level in the peripheral blood following treatment. The points shown at the extreme left indicate the control or precount values. Total leukocyte and differential counts were made on all 39 animals just prior to the start of the experiment. The average (arithmetic mean) leukocyte precount value was 11,550, the range 7,850 to 15,820, and the average deviation from the mean 1,640. The average lymphocyte value was 6,800±640 (range 3,460 to 11,100), and the average neutrophil value was 4,450±470 (range 2,500 to 6,620). The neutrophil-lymphocyte ratio, therefore, was nearly 40 percent: 60 percent. Horizontal lines have been extended across the graph at the level of the control points in order that the experimental values can more readily be compared with the precount averages at the various times.

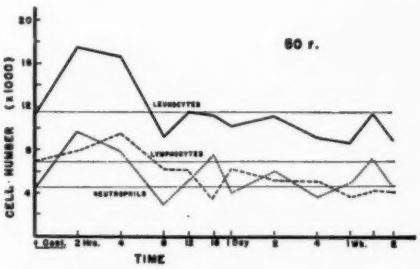

FIGURE 1.—Total leukocytes, lymphocytes, and neutrophils per cubic millimeter of peripheral blood following a single acute whole-body exposure of 50 r. Time is given on a log scale in order to show the early changes more plainly.

The leukocyte curve shows that a slight leukocytosis took place during the second to fourth hours. This was followed by a slight leukopenia within 24 hours which persisted beyond the period of the investigation. The lymphocyte curve follows the same general trend, but with the fall below the precount level somewhat more pronounced. The neutrophil curve shows a momentary initial rise followed by a fluctuation about the precount level.

It appears, therefore, that a dose of 50 r applied to the whole body of C3H mice induced a transitory leukocytosis which was followed by a slight but persisting leukopenia and that the leukopenia was due largely to a reduction in lymphocytes.

HISTOPATHOLOGY

Following a brief vasodilatation in all organs at the 2-hour interval, the earliest generalized tissue change noted was an increased eosin staining and mild cloudy swelling of the cell cytoplasm in the parenchymatous cells of many organs. The nuclei stained poorly. The relative increase in eosin staining was constant and was observed repeatedly. It occurred as early as 1 hour and ran a varying course depending upon the

[3] No attempt has been made to compensate for low-voltage radiation that may have failed to enter the ionization chamber during the first part of the starting cycle. However, since 0.5 mm. copper filter was used in all cases, it is apparent that the difference, if any, was inconsequential. The significance of this factor was reduced further by the fact that the shortest treatment interval used was several times greater than the starting cycle.

tissue. It disappeared not later than the twelfth hour. With disappearance of the cloudy swelling, the nuclei became slightly hyperchromatic but returned to normal in 24 to 48 hours. This whole change appeared to be a mild form of parenchymatous cell degeneration possibly induced or accompanied by a shift in pH of the tissues.

Definite destructive changes were seen in the lymphoid organs. At 1 hour after treatment, reticular cells of the lymph nodes showed prominently, and accumulation of these produced wedge-shaped sheets of cells about the periphery of the organs. Many of these cells became detached and were set free in the sinusoids. As early as 4 hours after treatment, multiple flecks of necrotic material appeared in the germinal follicles. These flecks were marked by small closely circumscribed groups of nuclear fragments (fig. 2, *A*). Multinucleated giant cells were present after 4 hours and as late as 3 days. Some of these cells contained as many as 40 nuclei (fig. 2, *B*). Between 8 and 12 hours the necrotic foci and the edema disappeared, giving way to an apparent increase in cellularity of the nodes. No clear mitotic wave was found although the 8- and 12-hour material suggested a slight excess of mitotic figures. The spleen showed changes similar to those in the lymph nodes together with an apparent increase in megakaryocytes. Pigment-containing macrophage cells were found in significant numbers at 18 hours. After this no other lymph-node abnormalities were noted except the occasional multinucleated cells which persisted through the third day. Similar, though less distinct changes were observed in the spleen.

Changes in the bone marrow were less marked and developed more slowly than did those of the lymph nodes and spleen. The earliest well-defined changes were seen in 8-hour material. Flecks of necrotic material such as those in the lymph nodes were not observed. Instead more adult myeloid series cells than normally were seen. This fact suggested that some slight but unnoticed destruction of immature cells had occurred at an earlier time. Megakaryocytes were abundant at 12 hours, and there was at this time an apparent increase in myeloblasts.

Under normal conditions, the mouse testis shows considerable variability in the state of activity from one section of the seminiferous tubules to another (*9*, *10*, *11*). As nearly composite a picture as could be gained of the experimental changes may be described as follows. There was a general cell swelling, as described for the other tissues at 2 to 4 hours, which gradually disappeared in 24 to 48 hours. Little else was seen until 1 week, when many of the tubules showed a reduction in the number of spermatogonia and primary spermatocytes (fig. 3). At 2 weeks some of the secondary spermatocytes had disappeared, and there was a definite reduction in the germinal cells generally while the sertoli cells persisted. Because of the frequent variations in activity among the tubules, identification of mild changes in the cells would have been difficult if it had not been for the absence of primary spermatogonia at 1 week to 10 days. Such absence was never noted in nonirradiated material. Extension of this study in another experiment showed that at 3 weeks many tubules contained beadlike rows of spermatogonia along the base, few primary spermatocytes, and almost no secondary spermatocytes. This condition was interpreted as active regeneration, the absence of secondary cells being attributed to the fact that not enough time had elapsed to allow this stage to be reached in the regeneration process. Even at the most destructive phase, following 50-r exposures, many of the tubules showed normal spermatogenesis, and after 4 to 6 weeks there was no evidence of injury.

Changes in the intestinal mucosa were mild but definite. Swelling occurred as early as 2 to 4 hours and was characterized by distinct hydroptic changes

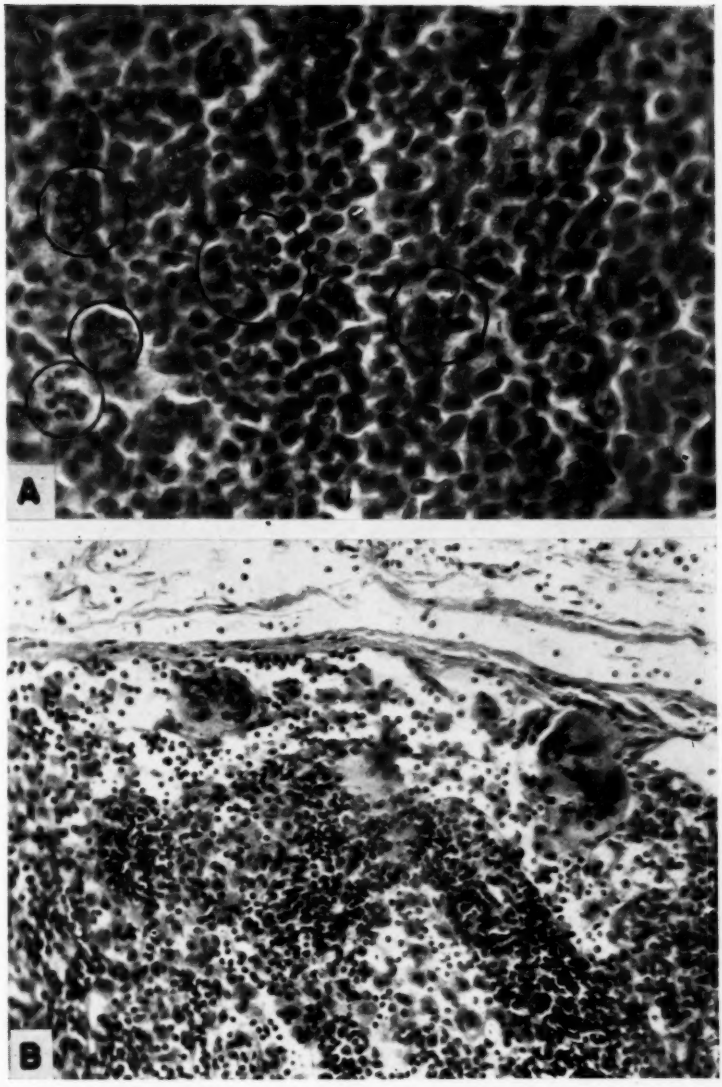

FIGURE 2.—Lymph-node sections. *A*, Four hours after treatment. Note the flecks of necrosis (circles) showing nuclear fragments and intact but apparently dead nuclei. × 475. *B*, Twenty-fours after treatment. Edema is marked; the large reticular cells are prominent, many lying free in the sinusoids. Note the sinusoidal packing with lymphocytes and the two large multinucleated giant cells beneath the capsule. × 200. Hematoxylin-and-eosin stain.

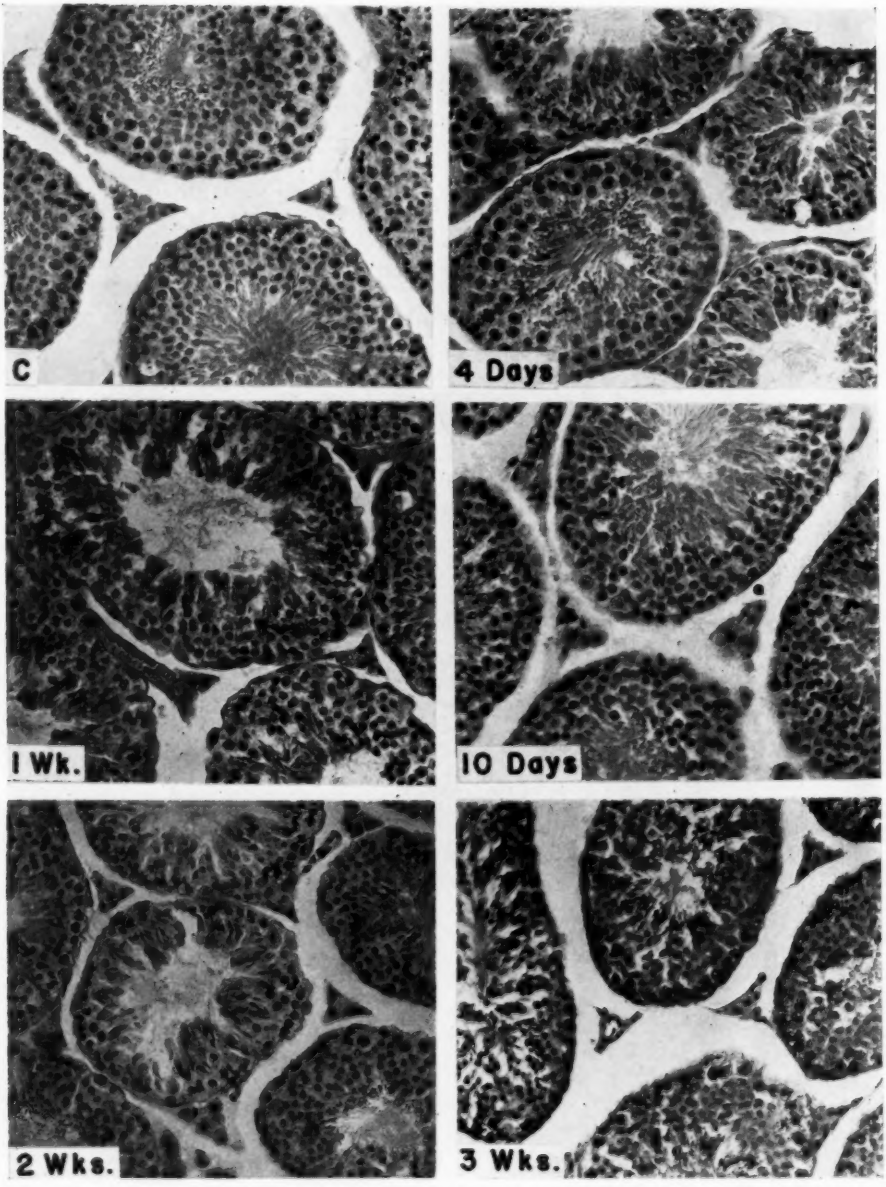

FIGURE 3.—Seminiferous tubules of the testis. C, Control, nonirradiated testis. The other index figures indicate the time after treatment when the tissues were fixed. Note loss of spermatogonia and primary spermatocytes at 1 week and 10 days, and the loss of secondary spermatocytes at 2 weeks. Evidence of regeneration may be seen at 3 weeks. Hematoxylin-and-eosin stain. × 200.

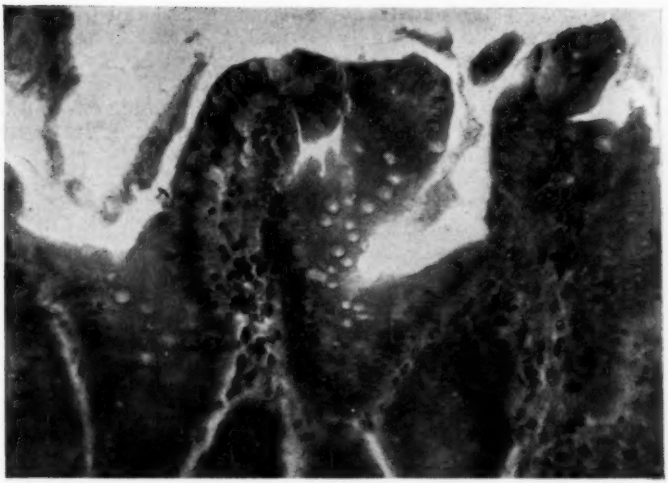

FIGURE 4.—Mucosa of the ileum fixed at 2 hours following irradiation. Note the smoothness of outline of the villus and the general cloudiness of the cells. Hematoxylin-and-eosin stain. × 200.

in the surface cells of the villi. Cells lining the deeper crypts appeared to be less effected (fig. 4). The swelling produced smoothly rounded surfaces on the villi, and the cells involved became vague in outline, showing acid staining of the cytoplasm and less distinct nuclei. The edematous condition and abnormal staining disappeared in 8 to 12 hours.

DISCUSSION

Early and systematic examination of the tissues of animals which had received 50-r whole-body treatments with X-rays revealed that slight but well-defined and widespread changes were produced. For the most part, these changes consisted of faint cloudy swelling, eosinophilic staining, slight tissue necrosis, and a small shift in peripheral blood picture. These modifications varied both in time of occurrence and in duration. From these findings, it is clear that, for the dose of radiation applied, the time of response is determined largely by the cells or tissues concerned and that the responses take place to some extent independently of each other.

Changes in the peripheral blood were detected as early as tissue injury, and the first blood response was a transitory leukocytosis. These observations suggest that the leukocytosis is an inflammatory response to tissue damage but that the higher leukocyte level could not be maintained because of destruction of various leukocytic components. The further fact that a sustained leukopenia was produced suggests that the limits of the leukocyte reserve were exceeded. Further evidence along this line is given in the paper following (*12*).

SUMMARY

Tissue and blood changes were systematically followed in C3H mice after the application of a single acute 50-r dose of X-rays to the whole body.

A slight leukocytosis developed in 2 to 4 hours, which was followed within 8 to 12 hours by a mild but persisting leukopenia. The leukopenia was found to consist mainly or entirely of lymphopenia.

A small amount of nuclear debris was seen in the lymph nodes at 2 to 4 hours. This nuclear debris disappeared, and a mild hyperplasia developed in 8 to 12 hours.

The seminiferous tubules of the testis showed a significant reduction in spermatogonia and primary spermatocytes at 1 week, and of secondary spermatocytes at 2 weeks. Regeneration took place, and the tubules appeared normal again in 4 to 6 weeks.

Other changes of lesser nature were also seen.

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EXPERIMENTAL ROENTGEN INJURY. II. CHANGES PRODUCED WITH INTERMEDIATE-RANGE DOSES AND A COMPARISON OF THE RELATIVE SUSCEPTIBILITY OF DIFFERENT KINDS OF ANIMALS

By PAUL S. HENSHAW, *senior radiobiologist, National Cancer Institute, National Institute of Health, United States Public Health Service*

INTRODUCTION

In the preceding paper (*1*), it was shown that acute roentgen doses of 50 r applied to the whole body of C3H mice produced a slight but distinct tissue injury in a number of organs. On the scale of therapeutic doses for human beings, 50 r is relatively very low. Such a dose is considered to have only the mildest therapeutic effect, if any (*2, 3, 4*), and by some (*5, 6*) as having a possible stimulating or tonic effect rather than a destructive one. Moreover, doses several times 50 r may be applied (locally) to patients in the case of fluoroscopic gastrointestinal examination (*7*). (See also (*8, 9, 10, 11, 12, 13*).) As the next step in the proposed study, it was important to determine the nature and extent of changes produced by larger doses.

The present paper deals with effects produced by doses commonly used in therapy ranging from 50 to 400 r. Again tissue morphology and the peripheral blood picture were used as indices of effect, and the examinations were carried out on a time basis. In this study, comparisons were made of the changes in two strains of mice and in one strain of guinea pigs.

MATERIALS AND METHODS

C3H male mice of the same inbred strain as those used in the first experiment (*1*) were used as one strain of mouse material, and LAF_1 animals as the other. The latter were F_1 hybrids of the inbred L and A strains maintained at the National Cancer Institute. The guinea pigs used were from a pure-bred stock at the National Institute of Health.

The dosages used were 50, 100, 200, and 400 r for each type of animal. Treatments were given to the mice in the manner previously described except that the distance was 50 cm., making the intensity 38.0 r per minute and the exposures 1.32, 2.63, 5.26, and 10.5 minutes, respectively, for the doses mentioned. The guinea pigs, being larger animals, were treated in pairs. During treatment each pair was confined in a light-weight, close-fitting cardboard box. Since the dorsoventral thickness of the guinea pig's body is 4 to 5 times greater than that of the mouse, 2 X-ray beams were employed to insure more uniform distribution of the radiation. One beam was applied from above and the other from below simultaneously. The distance from the center of the targets of the X-ray tubes to the middle of the animals in each case was 50 cm.; the voltage, current, and filtration were the same as used previously. The intensity was approximately doubled by using 2 tubes, being actually 71 r per minute as measured by the Victoreen instrument. The exposure times, therefore, were 0.70, 1.41, 2.82, and 5.64 minutes for the 50-, 100-, 200-, and 400-r doses.

HEMATOLOGIC CHANGES

Blood counts and tissue samples were taken at various times after treatments as described previously, except that two animals from each experimental group were used for blood counts instead of three. The peripheral blood changes produced in C3H mice by 50-r exposures have been shown satisfactorily (*1, fig. 1*). As indicated, the leukocyte fall was slight, consisted mainly of lymphopenia, and lasted more than 2 weeks. Changes produced by 100 r under the same conditions are shown in figure 1 of this paper. Here, the effects were more marked. As before, a slight leukocytosis was displayed within a few hours after treatment, followed by a

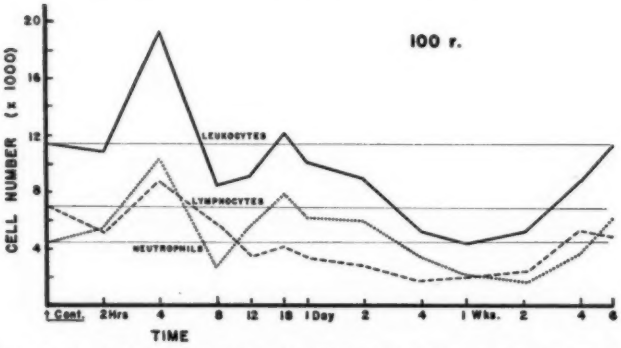

FIGURE 1.—Changes in leukocyte number in C3H mice after a single acute dose of 100 r applied to the whole body. The abscissas show time on a logarithmic scale and the ordinates absolute cell number per cubic milli meter of whole blood. The horizontal lines represent the average levels for precount con trols in each case.

leukopenia which became greater during the first week. The leukopenia in this case involved neutropenia as well as lymphopenia, although the lymphopenia developed more rapidly. During the second week, recovery began in both the lymphoid and myeloid elements, normal levels being reached during the period of the fourth to the sixth weeks. It was thus apparent that although the degree of leukopenia was greater for the 100-r dose than for the 50, the maximum effect was reached at about the same time and that recovery began no sooner in the case of the lower dose treatments.

Figure 2 shows the results obtained with LAF_1 mice receiving doses of 100, 200, and 400 r. Although data were obtained for 50-r doses, none of them are given since only the faintest suggestion of blood-picture change was noted. Thus, at the outset, evidence was obtained which suggested that this strain of mice was more resistant to X-rays.

The neutrophils in LAF_1 control mice were pitched at a significantly lower level than in the nontreated C3H mice, and correspondingly the lymphocytes were pitched at a higher level. The percentage ratio of lymphocytes to neutrophils in the former averaged 85 to 15, respectively, and that for the latter 60 to 40. Figure 2 (100 r) shows that a mild leukocytosis occurred during the first 2 to 4 hours, and that this was followed by a mild leukopenia which lasted throughout the first week. As in the case of the C3H mice, the latter appeared to consist mainly or entirely of lymphopenia. Figure 2 (200 r) shows only slight evidence of leukocytosis during the period 2 to 4 hours. The leukopenia which set in immediately became fairly marked, reaching a minimum during the fourth day. Recovery followed, and the normal level was reached between the third and fourth weeks. As before, the leukopenia consisted mainly of lymphopenia.

A comparison of figure 2 (100 and 200 r) with figure 1 of the preceding paper and figure 1 of this paper, respectively, shows that about the same degree of effect was produced in C3H mice by 50- and 100-r doses as was produced in LAF_1 mice by doses of 100 and 200 r.

The animals that received a single acute dose of 400 r showed only leukopenia, which reached a minimum at 4 days. As previously, a normal level of recovery was attained at 3 to 4 weeks, and some overcompensation at 6 weeks. In this case the lymphocytes began a downward trend at once after treat-

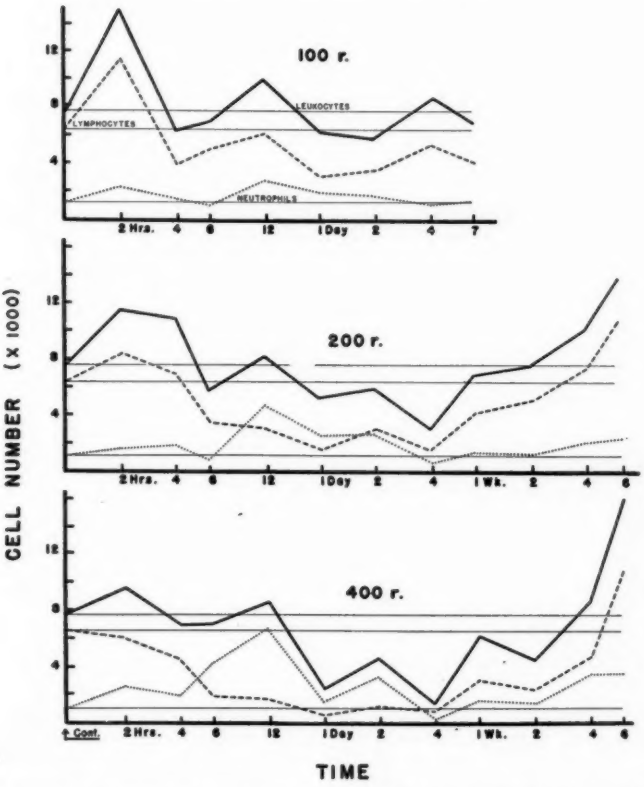

FIGURE 2.—Changes in leukocyte number in LAF$_1$ mice following a single acute dose of 100, 200, and 400 r, as shown. The abscissas show time on a logarithmic scale and the ordinates absolute cell number per cubic millimeter of whole blood. The horizontal lines represent the average levels for the precount controls in each case.

ment which continued through the fourth day, while the neutrophils developed transient but nevertheless significant rises during the first 3 days.

The three graphs of figure 2 form a progressive series. They show blood changes following a graded series of doses applied to one type of animal under comparable conditions. For each dosage used, the most conspicuous effect was lymphopenia, the amount of which varied directly with the size of dose. The time required for developing the lowest level of lymphopenia (4 days) and the time for recovery (4 weeks), on the other hand, was about the same irrespective of dose or degree of effect. Hence it is apparent that (for the conditions used) some features of the biologic response vary with the dose of radiation applied while others do not.

Figure 3 presents blood-picture curves for guinea pigs receiving 50, 100, 200, and 400 r. The values plotted in each case are based on averages obtained continuously on two guinea pigs. In two cases (100 and 200 r) one mem-

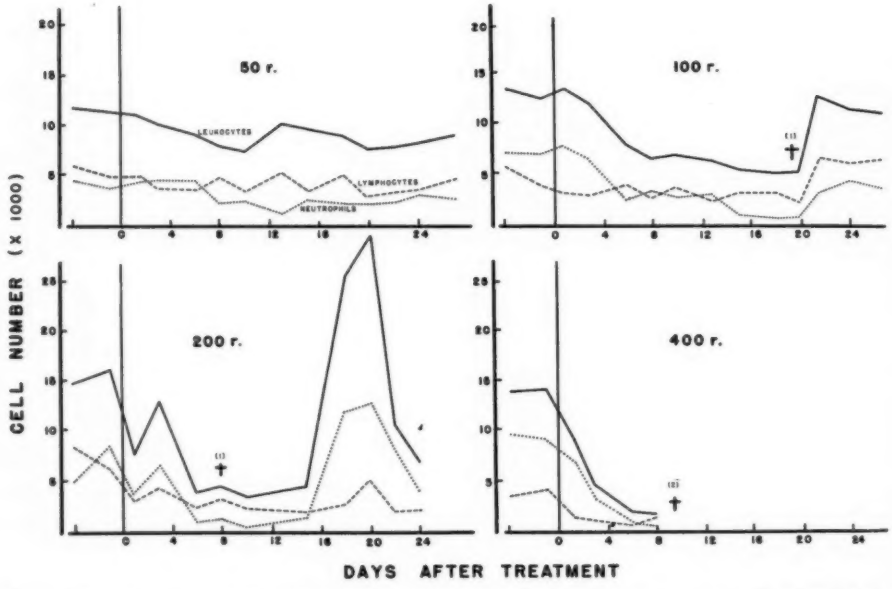

FIGURE 3.—Changes in leukocyte number in guinea pigs after a single acute dose of 50, 100, 200, and 400 r, as shown. Time is shown on a regular scale. Zero indicates when the exposures were made, and points to the left of zero indicate pre-irradiation counts on the same animals. Crosses (+) indicate deaths of animals.

ber of the pair of animals died several days ahead of the other; the later points are therefore based on counts for the remaining animal. In the precounts, the neutrophil and lymphocyte levels, unlike those in either the C3H or LAF_1 mice, were both pitched at about 50 percent.

The animals receiving 50-r doses (fig. 3) showed a mild leukopenia which reached a minimum at about 10 days. In contrast with the findings in mice, the leukocyte fall was due as much to neutropenia as to lymphopenia. The same general picture was obtained for animals receiving 100 r, except that the effect was greater. In this case the neutrophil level nearly reached zero at 15 days, and 1 animal died at 20 days. The other animal made a quick partial recovery and survived beyond the period of observation. In the case of the animals that received 200 r, the fall in leukocyte level again consisted of lymphopenia and neutropenia, the latter becoming less than 1,000 cells during the sixth day after treatment. One animal died on the eighth day. The other lingered, with a low leukocyte level, until the seventeenth day when it showed a spectacular rise in neutrophils and monocytes. The animals receiving 400 r developed marked leukopenia involving both lymphoid and myeloid elements in 4 to 5 days. Both animals died on the eighth day.

Erythrocyte counts and hemoglobin determinations were made on the different animals although not so frequently. Doses up to 400 r produced only occasional slight changes, if any, in the red-blood elements of the two strains of mice. Such changes, when seen, consisted of a very mild anemia at 7 to 10 days; recovery was rapid and complete. Guinea pigs likewise showed

little change in erythrocyte level except in those animals receiving lethal doses; in these varying degrees of anemia (acute microcytic hypochromic type with actual erythrocyte reduction and lowered hemoglobin) were seen just prior to death.

Changes in cell type were noted in the leukocyte series in both types of animals. In mice, particularly those receiving higher doses, more immature forms were seen. These were probably of the myeloid series since many band cells were found. In guinea pigs which received 100 r or more, the leukocytes appeared shrunken and contained deep-staining nuclei. Kurloff bodies and blast forms were frequently observed. In these animals just prior to the terminal stage, erythrocytes showed marked fragmentation in the smears. Thus for the same dose of radiation, it is apparent that guinea pig blood was affected more than that of the mouse, and further that the two types of mouse blood were affected about the same.

LETHAL DOSES

It has been indicated already that guinea pigs will succumb to doses of radiation that are not fatal to mice. Through the variety of experiments carried out, some idea has been obtained of the relative amounts of radiation the different types of animals can withstand.

Occasionally a guinea pig died within 7 to 10 days after receiving a single acute dose of 100 r applied to the whole body. More than half were killed by 200-r doses, and none survived 400-r. C3H mice were rarely killed by doses of 300 r, 20 to 40 percent were killed by 400 r, and all were killed by doses of 600 r. All LAF_1 mice survived doses of 400 r. The approximate lethal doses applied acutely to the whole body may therefore be listed as follows:

Guinea pigs	200 r
C3H mice	450 r
LAF_1 mice	above 450 r (probably about 600 r)

POST-MORTEM EXAMINATION

Enough animals of the different types and for the different dosages were used so that autopsies could be performed and tissues fixed at different times after treatment. The timing intervals varied with the type of animal and according to the rate at which changes occurred.

Gross examinations revealed very few changes. No modifications were seen in mice receiving 200 r or less. At 4 and 6 weeks after treatment, the fascia and connective tissue in those receiving 400 r appeared dehydrated and more fibrous, and the testes and lymph nodes in some cases were smaller than usual.

No changes were seen in guinea pigs that received 50 r or less. In those that died as a result of exposure, there was considerable congestion of the lungs, petechial hemorrhages and superficial ulceration in the gastrointestinal mucosa, atrophy of lymphoid tissue, and a gelatinous type of bone marrow. In the animals that received 400 r, the same but more extensive changes were seen. Petechial hemorrhages were widespread and showed on most of the visceral organs, abdominal wall, skin, etc. The tissues generally had a pinker cast. The liver in many animals was yellowish, with fine dark flecking.

HISTOPATHOLOGY

Tissues from the various animals were fixed at different times; Zenker's formol solution was used as the fixing fluid; paraffin sections were cut at 7 mm.; and hematoxylin-and-eosin stains were used throughout. As a rule, the following tissues were taken: lymph nodes (inguinal, axillary, cervical, and intestinal), spleen, bone marrow (sternum, spinal vertebra, femur), testis, intestinal mucosa, lungs, liver, kidney, heart, and adrenal.

Records were made of some of the more outstanding changes seen following the different dosages and at different times up to 6 weeks after treatment. For the present purposes, descriptions

are limited mainly to changes in the hematopoietic organs and testis, as these organs are of primary importance in problems of irradiation injury, and as the hematopoietic organs are probably the seat of changes leading to radioleukemia. Changes in LAF_1 mice are described in detail, and significant points of difference observed in C3H mice and guinea pigs are indicated. LAF_1 material was selected for detailed description only because it was a little more suitable for photographic purposes.

LYMPH NODES AND SPLEEN

Figure 4 shows lymph-node material at different times after exposure to a dose of 200 r. Within 2 hours, evidence of nuclear fragmentation was seen in the region of the germinal centers of the follicles. From subsequent conditions, it appeared that this effect was produced by the destruction of small (free) lymphocytes. At 4 hours, the cellular debris was evenly scattered, whereas at 12 hours it was more closely packed, indicating ingestion by macrophages. At 24 hours the debris was entirely cleared, and the field showed a distinct relative increase in reticular cells (fixed lymphocytes). At this stage, few or no dense areas of small lymphocytes could be found, while at 48 hours some such areas were observable. Recovery was slow, taking place over a period of many days. At 6 weeks the nodes looked essentially normal, although at this time there was some question whether relatively more reticular cells were present than normally.

Similar changes of lesser degree were seen in the 100-r material, and in 50-r material only the slightest evidence of necrosis was observed. Actually, no necrosis was found in the 50-r material except occasional small areas in Peyer's patches of the intestine.

The changes produced by 400-r exposures (fig. 5) were similar to those produced by 200 r except that the amount of destruction was greater. Recovery took place, as in the case of the lower doses, but here again there was a question whether fully normal nodes had been restored at 6 weeks.

General changes in the spleen resembled closely those seen in the lymph nodes (karyorrhexis, phagocytosis, and regeneration). Only slight evidence of damage was found in 100-r material, and none in the 50-r.

BONE MARROW

Bone-marrow changes following 100–r exposures are shown in figure 6. Loss of marrow cells began as early as 12 hours and continued through the first day. At 48 hours, relatively more blast cells were present, indicating the beginning of recovery. At 4 days, recovery was well under way, and at 1 week, the marrow appeared normal again.

Little or no cell loss could be detected in the marrow of animals receiving doses of 50 r.

Figure 7 shows the marrow changes following 200-r exposures. Some cell loss was apparent at 12 hours, and at 24 hours it was extensive. At 48 hours only a few marrow cells were present. At this stage, however, the remaining cells were reoriented and showed signs of regeneration, and at 4 days active regeneration was in progress. At 1 week recovery was approaching completeness although there appeared to be more than the usual portion of cells with ring-shaped nuclei present.

Changes folowing 400-r exposures (fig. 8) were still more extensive and lasted longer. Some cell loss was seen as early as 4 hours. Cell loss and necrosis continued for about a week, at which time the marrow spaces were nearly empty and the remaining cells in a damaged condition. Considerable recovery had occurred at 2 weeks, but the marrow was abnormal. The primitive cells were smaller than usual, and there were relatively more adult-type granulocytes. Further regeneration occurred, but it is not clear whether the marrow ever becomes entirely normal after 400-r doses.

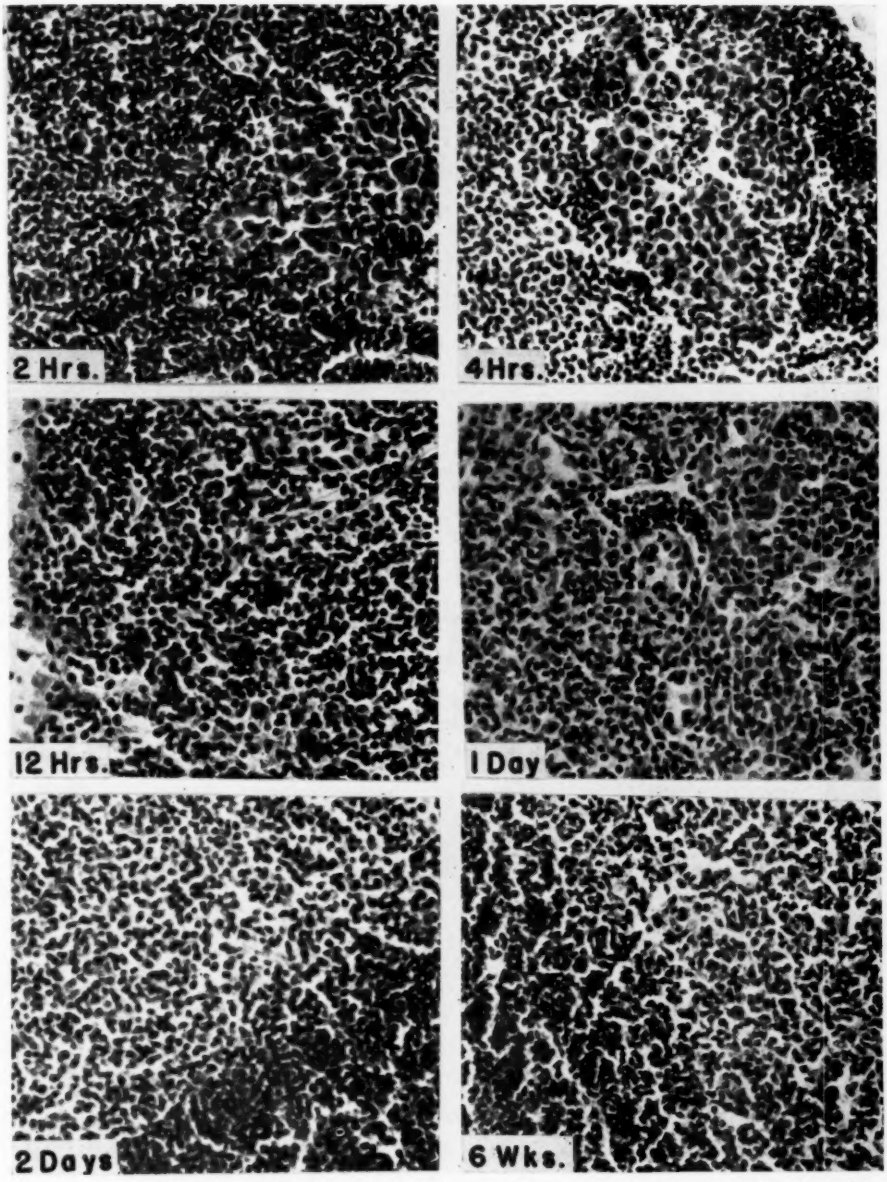

FIGURE 4.—Lymph-node material fixed at different times after treatment, following 200-r doses of X-rays. × 335.

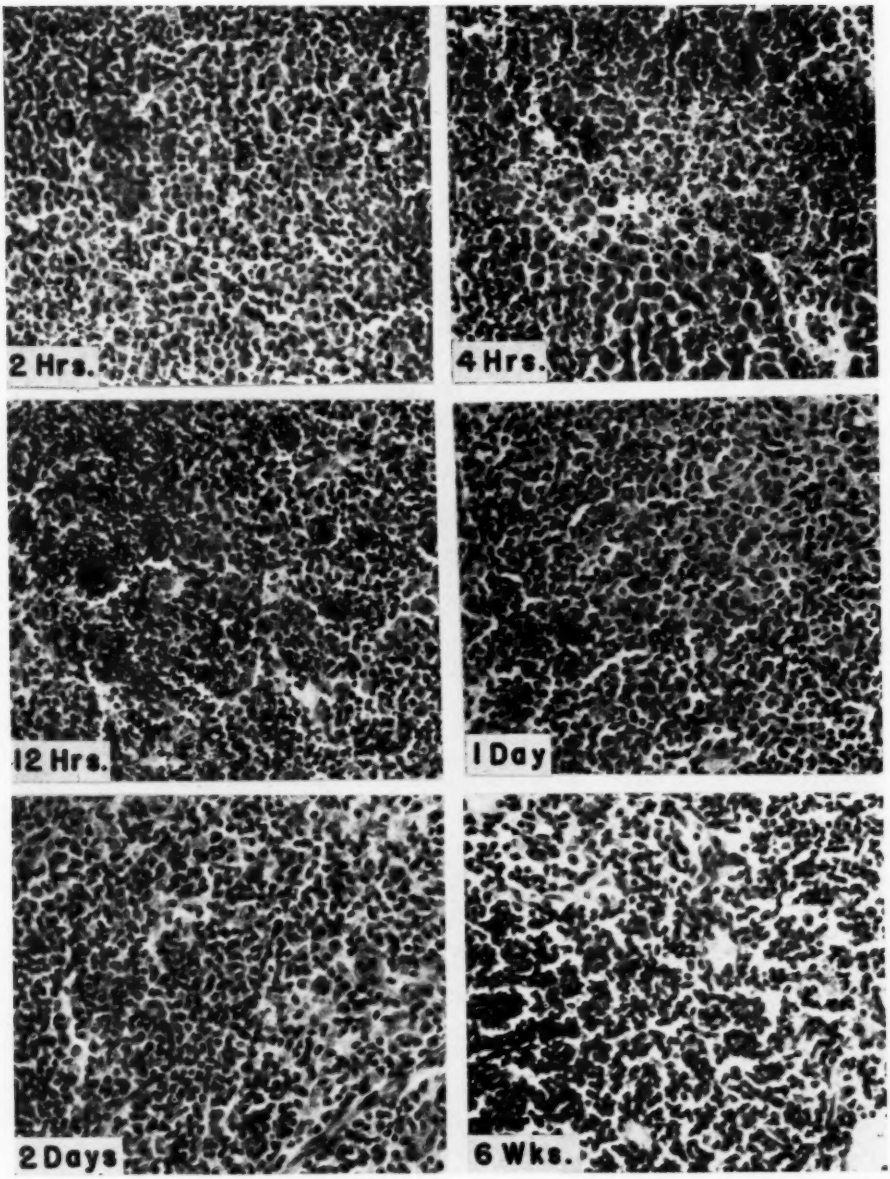

FIGURE 5.—Lymph-node material fixed at different times after treatment, following 400-r doses of X-rays. × 335.

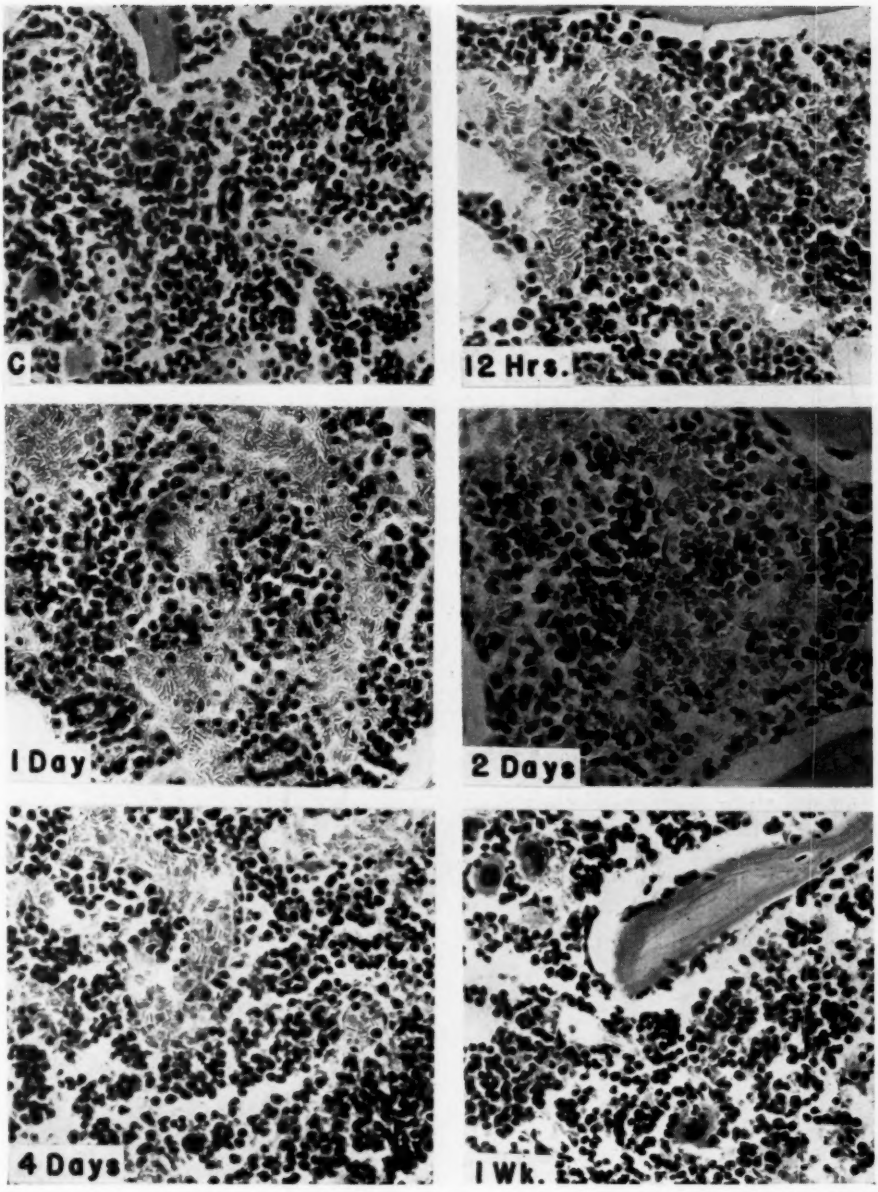

FIGURE 6.—Vertebral bone marrow fixed at different times after treatment, following 100-r doses of X-rays. × 335.

Femoral and sternal marrow were also examined at the different times after treatment and after the same exposures. The impression was gained that the degree of damage was always a little greater in the vertebral marrow than in the femoral or sternal.

TESTIS

It was pointed out (*1*) that C3H mice receiving 50 r showed little change in the testis during the first 4 days after exposure, that loss of spermatogonia and primary spermatocytes occurred during the last part of the first and during the second week, that this was followed by loss of secondary spermatocytes and sperm, and finally that regeneration was in progress during the third and fourth weeks, apparently becoming complete soon thereafter. Much the same cycle of change was seen in LAF_1 mice receiving 200 r (fig. 9).

LAF_1 animals receiving 100 r showed changes less severe than those in C3H animals receiving 50 r, and the LAF_1 animals receiving 50 r showed little or no perceptible change.

In LAF_1 animals that received 400 r, some evidence of denser staining nuclei and looseness of tissue architecture could be observed among the primitive cells at 2 to 4 days, and at 1 week (fig. 10) there was almost a complete loss of primary spermatocytes and some spermatogonia in most of the tubules. Attempts were made to find similar tubules in nonirradiated control material from animals of the same age and handling, but none were found. Some normal tubules showed a distinct loss of secondary spermatocytes but never of early forms. As further disintegration of primitive cells occurred and further maturation of secondary spermatocytes took place in the 400-r material, most of the tubules became almost devoid of germinal elements. Tubules at 4 weeks (fig. 10) contained few cells other than sertoli. When regeneration began, the tubules showed only primitive cells as no late stages had been reached as yet. This condition may be seen best in figure 9 (6 weeks). Whether the new germinal elements arose from occasional remaining germinal cells or immigrated from other areas was not determined. Even after 400-r exposures, some apparently normal tubules were observed at nearly all stages.

C3H MICE AND GUINEA PIGS

Thus far the histopathologic descriptions have pertained largely to LAF_1 mice. The material for C3H mice and for guinea pigs for similar doses and times were also examined. It has been indicated already that the lethal dose was least for guinea pigs, intermediate for C3H mice, and greatest for LAF_1 mice. In a general way, the tissue damage sustained varied in the same manner. For equal amounts of radiation applied, decreasing amounts of tissue damage were seen in guinea pigs, C3H mice, and LAF_1 mice, in the order given. Other quantitative and qualitative differences were seen; but these were of detailed character; and since we are here more concerned with the over-all picture, no further descriptions will be given.

DISCUSSION

Generally, the same kinds of changes were observed in the peripheral blood, lymph nodes, spleen, bone marrow, and testis in C3H mice, LAF_1 mice, and guinea pigs, following doses of ionizing radiation in the range 50 to 400 r. These changes consisted in a loss of functional cell elements followed by a slow return to normal if the damage had not been too severe. Closer comparison of the changes, however, revealed important differences as well as similarities.

A fall in lymphocyte level occurred uniformly in all three groups of animals following exposure, and for the range and dosage used the period during which the fall occurred was generally the same in each kind of animal (4 days to 1 week). On the other hand, the amount of fall for any given dose varied, the guinea pigs showing the greatest

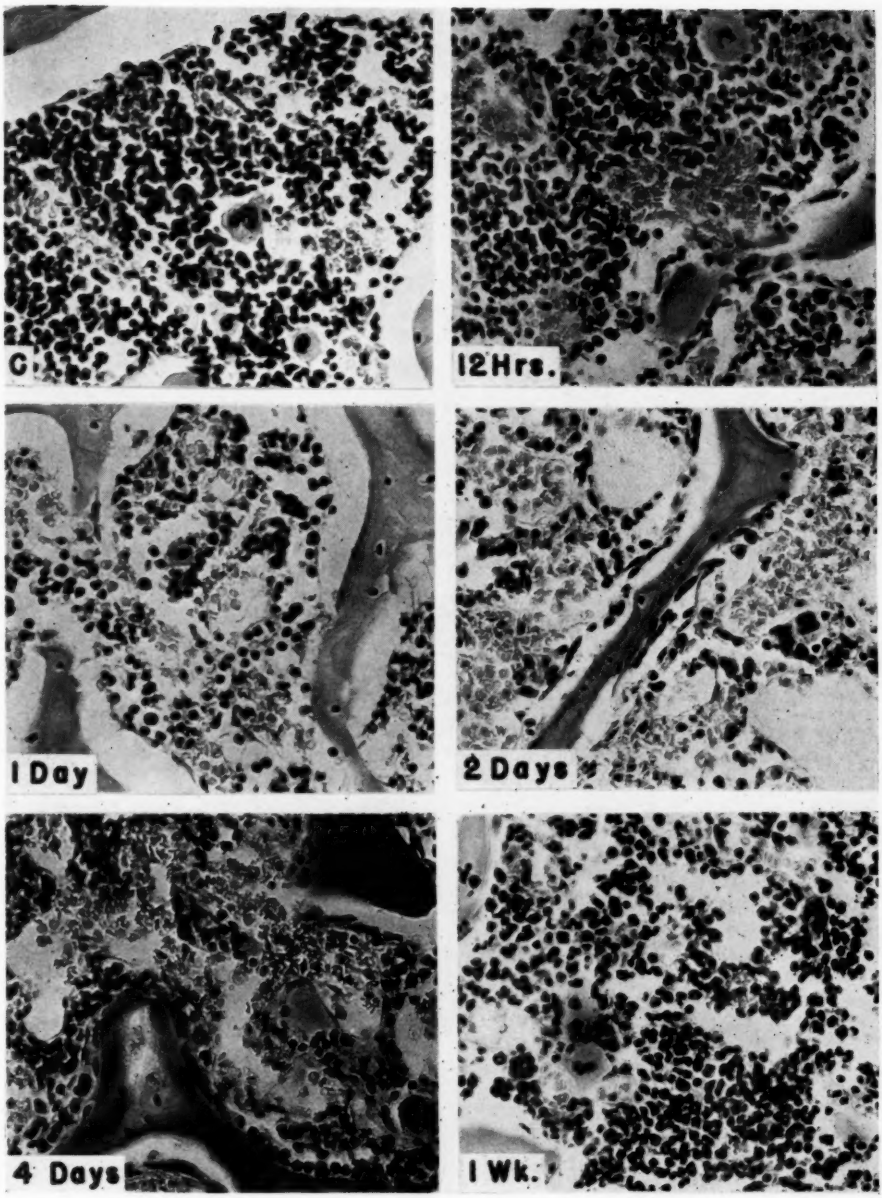

FIGURE 7.—Vertebral bone marrow fixed at different times after treatment, following exposure to 200-r doses of X-rays. × 335.

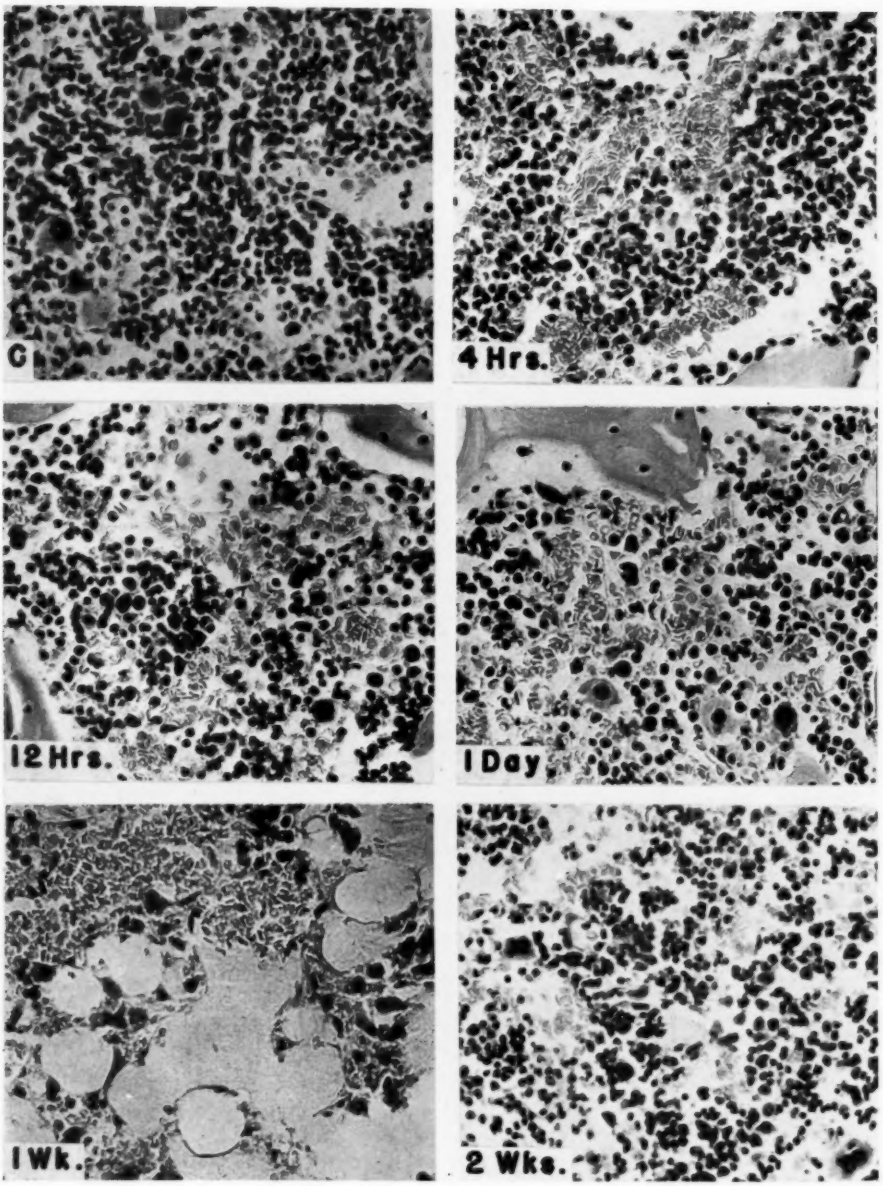

FIGURE 8.—Vertebral bone marrow fixed at different times after treatment, following 400-r doses of X-rays. × 335.

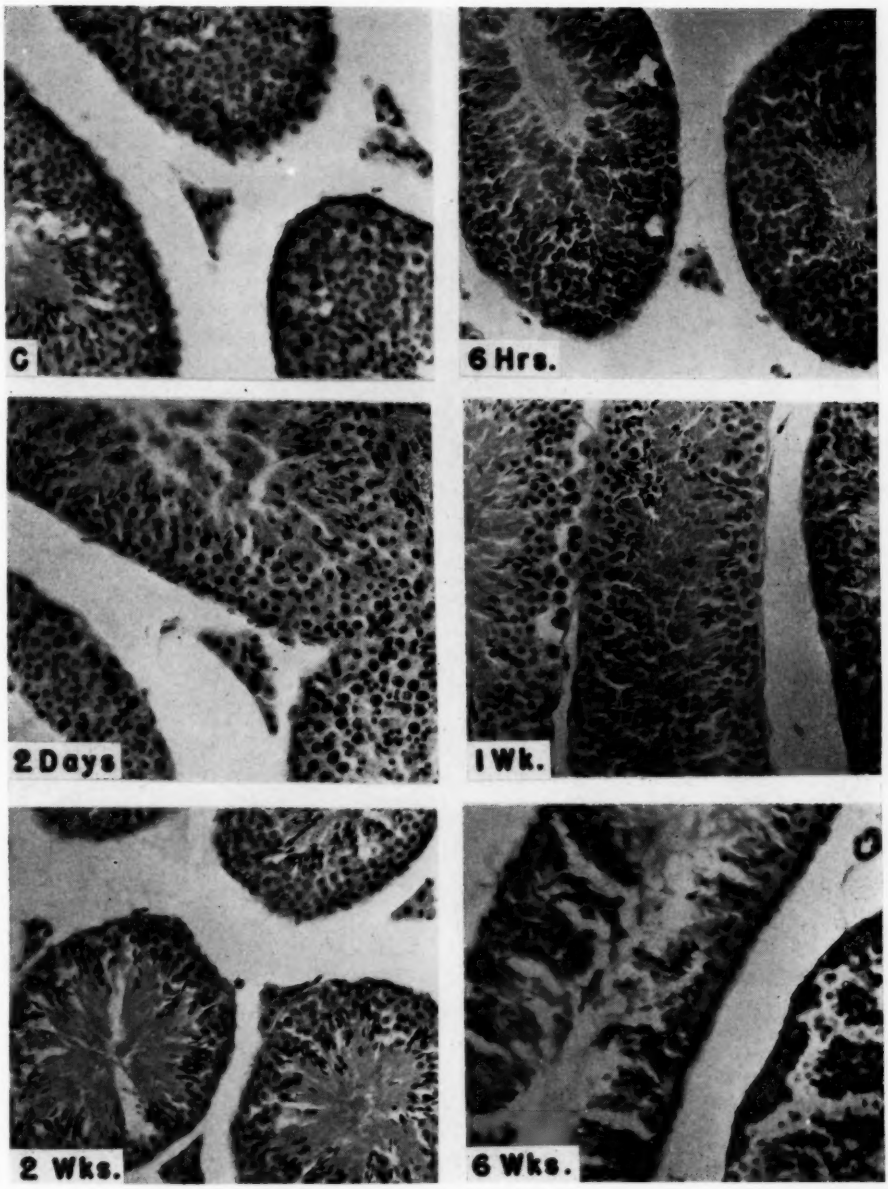

FIGURE 9.—Testis material fixed at different times after treatment following doses of 200 r. × 260.

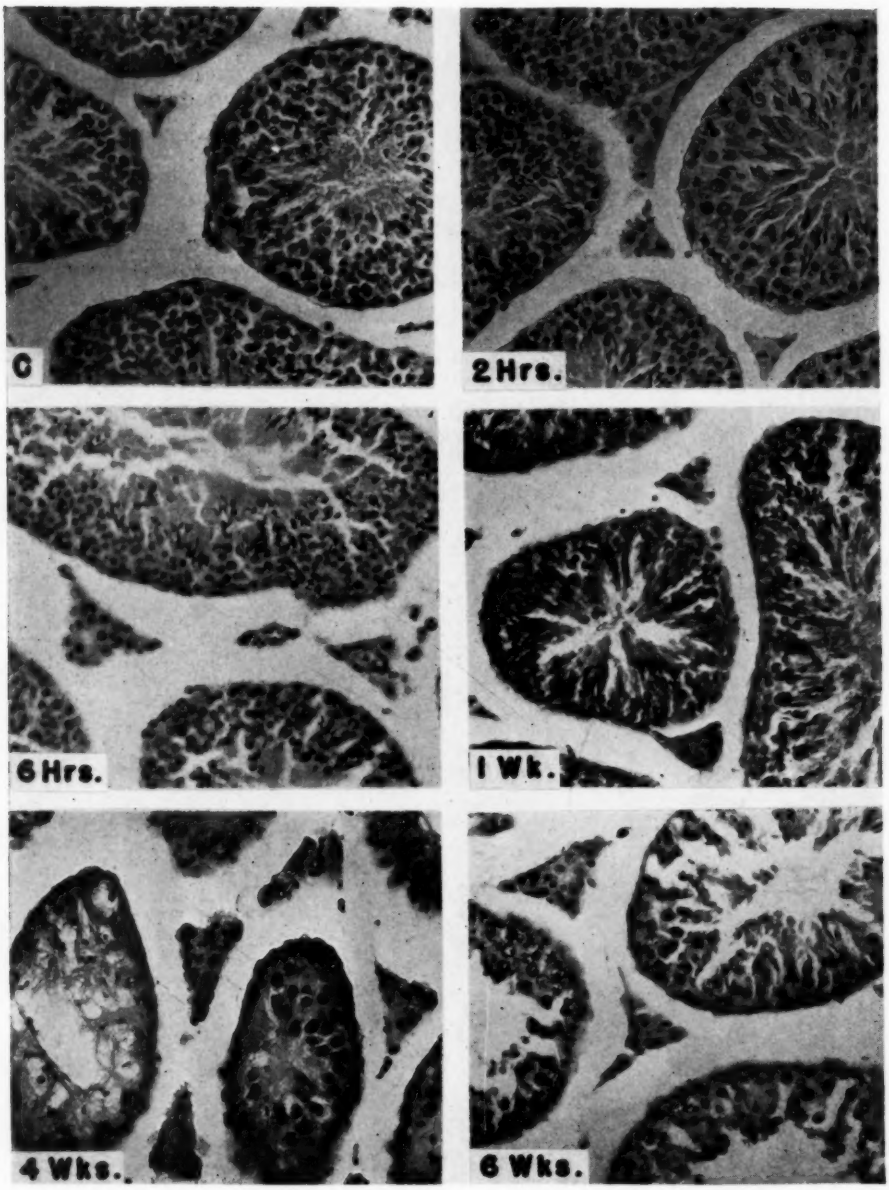

FIGURE 10.—Testis material fixed at different times after treatment following doses of 400 r. × 260.

fall and LAF_1 mice the least. A fall in neutrophil level likewise was observed in the three groups of animals, but it was not the same in each case. In guinea pigs, the neutrophils showed about the same susceptibility as did the lymphocytes, the response in each case running closely parallel. In the C3H mice, the neutrophil response began later than that of the lymphocytes, although the lowest level was reached at about the same time. The neutrophil fall in LAF_1 mice was preceded by an unstable but distinct rise in neutrophil number, but here also the lowest level for neutrophils and lymphocytes was reached at about the same time.

Histologically, all lymphoid tissues manifested essentially the same sequence of changes, namely, loss of small lymphocytes within 24 hours after exposure resulting in a closer association of reticular cells, and a return to normal requiring 2 to 6 weeks or more. Bone marrow showed a similar loss of parenchymatous tissue, but because of the fixed character of the marrow cavities the remaining cells could not shrink together in the same manner as in lymph nodes. Thus, the loss of marrow cells resulted in the formation of extensive blood sinuses. The period during which cell disintegration took place was longer in the case of bone marrow, lasting up to 4 days or more. Recovery of marrow tissue, like that of the lymphoid tissue, extended over an indefinite period, as much as 6 weeks or more. Accumulations of nuclear debris did not show in the marrow tissues a few hours after treatment as in the lymphoid, but from the fact that a greater proportion of mature cells showed in the marrow within 6 to 24 hours after treatment, it may be presumed that there was a corresponding disappearance of younger cells soon after treatment. The cell type, which finally remained in the marrow spaces and from which marrow tissues must have arisen, appeared to be primitive in character and not unlike the reticular cells which remained in the lymphoid organs.

Normally the germinal elements of the testis are arranged more in the order of maturation, thus making the developmental stages easier to study. Careful examination of the experimental material disclosed that the primary spermatocytes were the first to disappear following irradiation. When intermediate-range doses were administered, sections were obtained which showed mainly a loss of primary spermatocytes at 1 week. With higher doses, the seminiferous tubules showed complete loss of primary spermatocytes and a distinct thinning of spermatogonia. As time passed, the secondary spermatocytes disappeared, owing in part or entirely to further maturation. As a consequence, the tubules became empty, apparently because there were no primary spermatocytes to be transformed into secondary spermatocytes. Regeneration took place by the cell types reappearing in the order of maturation, that is, spermatogonia alone first, then spermatogonia and primary spermatocytes, and finally all three cell types, with the eventual appearance of mature sperm. This fact suggests that the extensive loss of cells from the testis resulted primarily from the destruction of one cell type, the primary spermatocytes. It further suggests that some of the more primitive cells were able to withstand doses of radiation that were fatal to primary spermatocytes, in this respect contradicting the view generally held that the more primitive the cell type, the more vulnerable to radiation.

If it be assumed that the reticular cells of the lymphoid organs and the residual cells of the bone marrow are parent forms, and also that the small lymphocytes are an intermediate stage between parent cells in the lymphoid organs and the variety of more specialized blood cells, an interesting parallel exists between the response of germinal epithelium of the testis and hematopoietic tissues.

It would appear, then, that the extensive cell loss in all cases resulted

from destruction of an intermediate-stage cell and the further maturation and disappearance of older cells which could not be readily replenished. The cell from which regeneration started in each case was a stem form, obviously more able to withstand irradiation injury than the cell just older in development. It is this primitive form which must act as the parent cell to both the normal or aberrant forms which may arise. Hence, the problems of the organism following moderate doses of radiation appear to depend in large measure on injury to stem cells. If large numbers are destroyed, sterility (in the broader sense) is likely to result, and if their competence is altered, various types of aberrant behavior may be manifested.

It was emphasized previously (*1*) that the peripheral blood picture did not follow closely the histologic picture in C3H mice following 50-r exposures and also that this failure appeared to be due to leukocyte reserve. The early leukocytosis observed tended to disappear with increasing doses, and, for the most part, to be entirely absent in the case of the more radiosensitive guinea pigs. This fact suggests that with an increase in dosage the destruction of leukocytes in the peripheral blood and in the hematopoietic centers soon exceeds the reserve of these cells with the result that a leukopenia quickly develops.

Numerous differences in types of response have been described for the various animals and tissues. These are sufficient to indicate the necessity of using homogeneous animal strains in critical studies and likewise the care that must be taken in dealing with discrepancies in results obtained with different types of animals.

SUMMARY

Doses of 50, 100, 200, and 400 r were applied to the whole bodies of C3H and LAF_1 mice and to guinea pigs. Studies were made of the hematologic and histopathologic changes following exposure. The histologic studies for the most part pertained only to the lymphoid organs, bone marrow, and testis.

Important differences and similarities were observed. In a general way, the tissue response consisted of a parenchymatous-cell loss followed by a slow return to normal if the damage was not too severe.

Loss of functional cells appeared to result both from destruction of certain primitive cells and from further maturation.

Regeneration in each case started from primitive cell forms that had survived irradiation fatal to other cells.

Leukocyte reserves appeared to prevent the peripheral blood picture from reflecting precisely the condition of the hematopoietic tissue when the dosage was slight.

Idiosyncrasies in the animal types were sufficient to indicate the necessity of using homogeneous groups of animals in making critical studies.

The problems of the organism following moderate doses of radiation appear to depend largely upon injury to the stem cells of regenerative tissues.

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EXPERIMENTAL ROENTGEN INJURY. III. TISSUE AND CELLULAR CHANGES BROUGHT ABOUT WITH SINGLE MASSIVE DOSES OF RADIATION

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INTRODUCTION

Descriptions have already been given of blood and tissue changes in mice and guinea pigs which followed the application of 50-to-400-r doses of X-rays (*1*, *2*). The changes that follow exposures of 50 to 100 times these amounts are considered in the present paper.

Heretofore death following irradiation had not been seen sooner than several days after exposure, and we desired to ascertain whether this period could be appreciably shortened. The very first test showed not only that it could, but also that further information concerning the nature and types of irradiation injury could be obtained by using massive doses. In the previous work with therapeutic doses, tissue destruction was the most prominent feature, clinical symptoms being relatively few. Single acute exposures were fatal in but one of the two species of animals used (guinea pigs). Injury to the organism as a whole may result not only from loss of cells and tissues following irradiation but also from tissue disintegration products. Various authors (*3–8*) have called attention to the fact that radiation sickness and even death may result from toxic products produced by cytolized tissue substances. This reaction has been considered a form of shock.

By increasing the dosage of radiation, the shock reaction was brought to the fore, making clinical symptoms prominent as well as tissue disintegration. The type of shock produced with massive doses (25,000 to 50,000 r) was so different from any previously described that in some respects it represents a new complex.

MATERIAL AND METHODS

Ten C3H mice, four guinea pigs, and twelve rabbits were used in these experiments. Larger numbers of animals were not used because the reactions were so definite that little was to be gained by doing so. Exposures were made in generally the same manner as that described previously. In all cases the two X-ray tubes were employed together with simultaneous cross-firing beams from opposite directions. When mice and guinea pigs were treated, the beams were directed vertically; but when rabbits were exposed, they were directed horizontally. The treatment conditions in all cases were as follows: 200 kv., 20 ma., no filter, 50 cm. distance (from the center of the target to the center of the test animals). The intensity at 50 cm. was 250 r per minute (as measured in air with the Victoreen instrument).

RESULTS

SYMPTOMS

Responses were much the same in all three types of animals. Hence descriptions for one apply in a general way for all, although certain details are given for each type of animal.

The first test was carried out on a pair of guinea pigs placed in the X-ray field. After exposure had been in progress about an hour, both animals became restless and showed signs of increased and labored respiration. Soon evidence of cyanosis was displayed and also a curious intermittent spastic twitching over the whole body. At about 90 minutes, when the accumulated dose of 25,000 r had been reached, one animal was removed from the X-ray field, treatment of the other being continued. During the next 90 minutes, periods of heightened activity alternated with periods of stupor and inaction in both animals. The second animal was removed from the field when the accumulated dose reached 50,000 r which was approximately 3 hours after treatment started. Both animals died at about the same time, within a few

minutes after the end of the longer treatment.

Rabbits, because of their size, were treated one at a time. Restlessness, labored respiration, and cyanosis likewise began in these animals after about 90 minutes' exposure, and periods of heightened activity were displayed during the second interval of treatment. Unlike the guinea pigs, however, the rabbits did not die until 3 or 4 hours after receiving the 50,000-r treatment. During the post-treatment interval some animals showed respirations over 165 and a pulse over 200 per minute. The temperature in some cases reached as high as 42.5°C. For a short time after treatment there appeared to be a lessening in the general body spasticity as the animal did not readily respond to the stimuli of cold or pinching. The eye reflex was diminished, and the eyeball was rolled back. It was found later, however, that these changes were due to the fact that the whole muscular system was in a state of mild spasticity. Strong stimuli would set off violent responses in the form of quick, uncoordinated movements. The forelegs and hind legs for the most part were in extension. Finally the animals died, lying on their sides in a state of muscular spasm. Animals receiving 25,000-r doses sometimes lived 24 to 48 hours, but the picture otherwise was generally the same.

Mice showed similar symptoms although not to the same degree as a rule. Labored respiration was not so plainly observed, and the state of excessive reactivity usually did not set in until after the treatments were completed. Unless disturbed, these animals were quiet and appeared exhausted. However, if the cages were disturbed or a sharp noise was heard, all the animals responded with periods of violent vertical jumping which sometimes lasted a half minute or more. Beginning soon after the treatment period ended, a slimy fecal material exuded almost continuously. Alternating between periods of stupor and hyperactivity, the animals lived for a number of hours. Those receiving 50,000 r usually died at about 10 hours after the beginning of treatments, whereas those receiving 25,000 r died after 24 to 48 hours.

GROSS POST MORTEM EXAMINATIONS

Post-mortem examination immediately after death of rabbits that had received 50,000 r revealed that the state of muscular spasm may have passed directly into rigor mortis. The hyperthermia previously noted was still prominent, the tissues being noticeably warm (one animal which survived almost 24 hours after a 25,000-r exposure had a temperature of 43.4° C. just prior to death). Blood withdrawn by cardiac puncture from the left side of the heart was dark purple. The heart itself was soft and not distended. The lungs were firmer than normal, but there was no evidence of edema. The spleen was small, dark purple, and very soft. A longitudinal section of the sternum showed the marrow to be gelatinous. The liver appeared to be normal. The gastrointestinal tract showed an edematous mucosa but otherwise appeared normal. There were no petechial hemorrhages. The kidneys were dark purple but otherwise appeared normal. The scrotum was dark purple, and the testes were small and soft. The brain showed a dusky purple color throughout the cortex.

BLOOD PICTURE

Table 1 shows blood-count figures which were obtained just before treatment and 1 and 2 hours after treatment started. Rabbit 4 had received a dose of 25,000 r applied to the whole body and rabbit 5 twice as much. The figures for hemoglobin and erythrocytes show that no significant change occurred during these intervals for either dosage. Those for the leukocytes on the other hand show changes resulting from both doses. During the first hour the number dropped to about a third and during the second hour (rabbit 5) to about a tenth. All cell types appeared to suffer alike. Normoblasts were frequently seen in the blood of animals receiving these treatments.

TABLE 1.—*Early effects of massive dose X-rays on the peripheral blood*

Blood elements	Rabbit 4 (25,000 r)			Rabbit 5 (50,000 r)		
	Hours after treatment began			Hours after treatment began		
	0[1]	1	2	0[1]	1	2
Hemoglobin.....grams 100 cc..	13.0		15.5	13.0	13.8	13.5
Erythrocytes.....millions..	5.9	6.39	6.48	5.32	5.17	5.28
Leukocytes.....number..	10,600	3,050	3,200	12,700	4,700	1,150
Neutrophils.....number..	4,240	790	1,460	2,030	890	70
Lymphocytes.....number..	4,880	1,950	690	8,380	3,200	550
Monocytes.....number..	530	60	320	370	90	20
Eosinophils.....number..	110	0	0	0	0	0
Basophils.....number..	330	0	0	130	190	70
Metamyelocytes.....number..	530	240	320	1,140	280	140
Myelocytes.....number..	0	0	0	250	50	20
Blasts.....number..	0	0	0	0	0	0
Normoblasts.....number..	0	0	0	0	0	[2]

[1] Just prior to treatment.
[2] Many seen.

Blood withdrawn from the heart (as previously described) was found to have a clotting time of 10 minutes. The clot when formed was very fragile and could be broken easily. Moreover, there was no clot retraction after 4 hours. Blood left standing in a tube showed no sedimentation after 50 minutes. The plasma coagulability test for prothrombin (Howell) was 8 minutes. The blood of animals which had survived as long as 24 hours showed some evidence of hemoconcentration (erythrocytes 7.81 million, leukocytes 8,700, hemoglobin 19.5).

MICROSCOPIC EXAMINATIONS

The devastation produced within the animal's tissues in the brief period of 3 hours was almost unbelievable. There was no tissue in the body which did not suffer; even the most resistant of tissues such as brain and muscle showed marked damage. Practically none of the cells failed to show evidence of injury. Many were so much affected that only nuclear fragments could be seen. The cells which remained intact invariably showed greatly swollen cytoplasm and nuclear or perinuclear edema.

LYMPHOID ORGANS

Lymphoid tissues were so disarranged and so disorganized that they gave the appearance of generalized tissue necrosis (fig. 1). Pyknotic and karyorrhectic nuclei with confluent masses of cytoplasmic debris were scattered throughout. The only cells which appeared sufficiently intact to have regrown if the animal had survived were large, swollen, pink-staining, primitive-type cells. Even the nuclei of these were greatly swollen and vesiculated. A similar but smaller type cell appears to have survived in lesser numbers.

BONE MARROW

Sternal and vertebral marrow were studied. All cells were swollen, and there were many aberrant nuclei (fig. 2). Scattered and fragmented nuclei were common. Here again the only cells which might conceivably have survived were large, swollen, pink-staining, primitive types. There appeared to be multinucleation of some of the primitive cells. The amount of actual cell debris seen was not so marked as in the case of lymphoid organs, but the amount of damage was probably as great. In some of the sections most of the bone marrow was replaced by fluid accumulations.

TESTES

Changes in the testes were extensive. Sections of a rabbit testis taken 4 hours after the end of a 25,000-r treatment showed cytoplasmic degeneration, nuclear darkening, and extensive cell loss (fig. 3, *A*). Cellular destruction progressed until at 36 hours only skeletons of tubules remained (fig. 3, *B*).

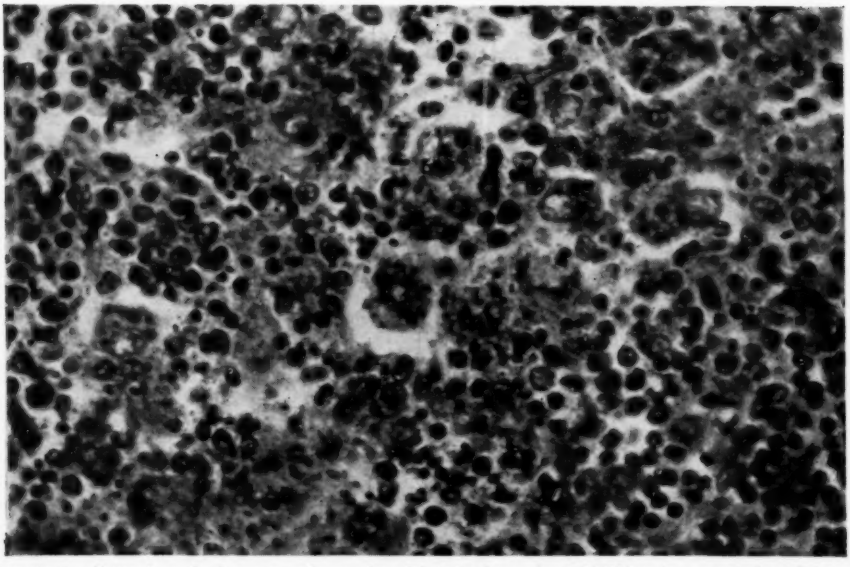

FIGURE 1.—Lymph node from a guinea pig which had received 50,000 r in a period of 3 hours and had died at termination of treatment. Immediate autopsy and fixation in Zenker-formol solution. Hematoxylin-and-eosin stain. × 1100.

OTHER ORGANS

The free edge of the mucosal cell of the small intestine was ragged following treatment, but in those animals which died immediately no evidence of mucosal sloughing was seen. Large numbers of inflammatory cells were infiltrating the submucosa and mucosa. Marked swelling showed in all cell elements. Edema of the submucosal connective tissue was extensive, and there was edema in all coats. Similar changes were seen in the large intestine, stomach, and cecum. The solitary lymph follicles in the large intestine showed extreme destruction.

A section of skin removed from the scrotum of a rabbit which had received 25,000 r locally to the testes (as explained later) revealed massive edema of the subcutaneous tissue and a marked perinuclear edema of the epithelial cells. The best preserved nuclei were found to lie in the stratum germinativum. Scattered inflammatory cells were present in the subcutaneous connective tissue.

The lungs in guinea pigs that died immediately at the end of treatment showed distended vessels with scattered capillary hemorrhages and occasional areas of fluid in the alveoli. Some areas of atelectasis were present, and leukocytes appeared in the alveolar walls. In animals that lived longer, congestion progressed with time until the alveoli became filled with a heavy, pink-staining material. In some cases, this congestion became severe (fig. 4).

Sections from the liver, kidneys, and adrenal showed some cellular edema and nuclear damage. Glomerular capillaries displayed irregular dilatation. Both striated and nonstriated muscle showed noticeable cloudy swelling and actual destruction of muscle substance with collapse of sarcolemma at some points. Blood vessel walls generally showed no distinctive alterations.

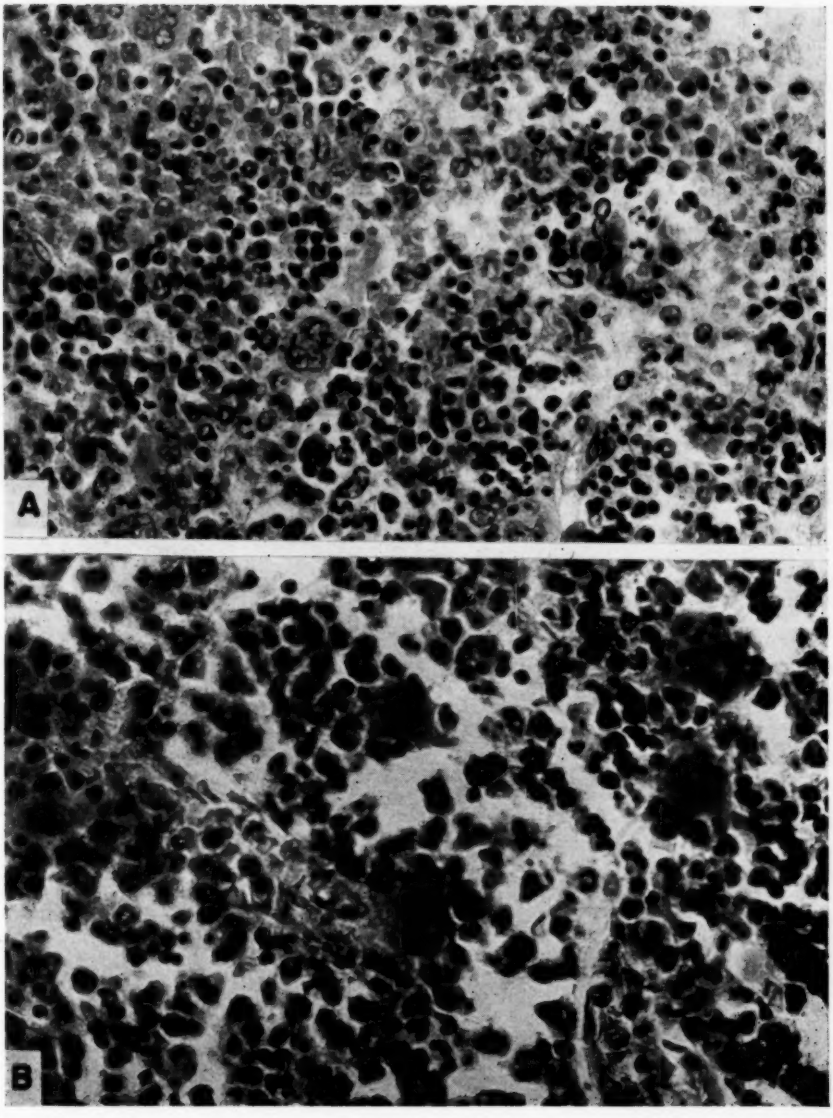

FIGURE 2.—*A*, Sternal marrow from a guinea pig which had received 50,000 r in a period of 3 hours and died at termination of treatment. × 220; *B*, Same. × 1100. Hematoxylin-and-eosin stain.

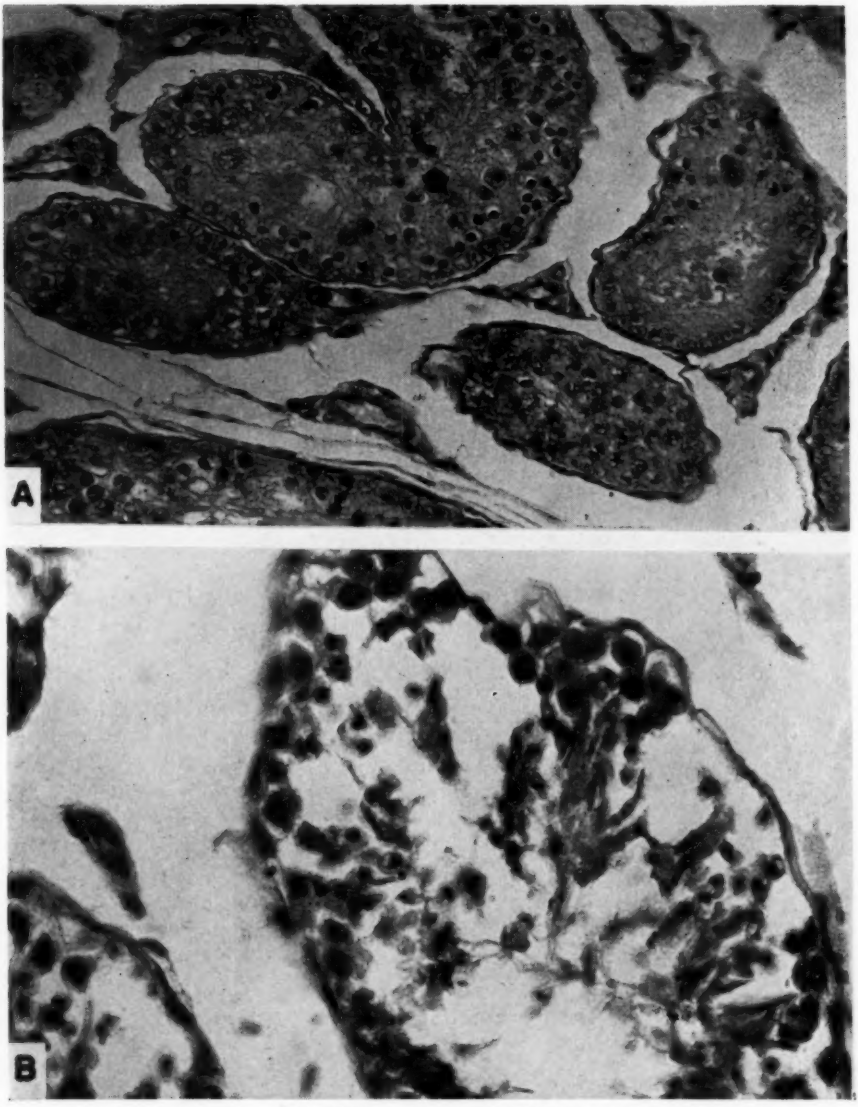

FIGURE 3.—*A*, Testis from a rabbit which had received 25,000 r and died 4 hours after the end of treatment. Note the cytoplasmic disorganization, edema, and extensive cell loss. × 260; *B*, Rabbit testis treated locally with 25,000 r and fixed at 36 hours after the beginning of treatment. This condition was obtained irrespective of whether the radiation was applied locally or to the whole body. × 1100. Hematoxylin-and-eosin stain.

Extensive changes occurred even in the nervous system, which is one of the parts most resistant to radiation. Glial and neuron cells of the brain showed a small amount of edema and some scattering of Nissl substance. The axones showed massive edema, with splitting of the sheaths. The ganglion cells displayed pericellular edema, swelling and overstaining of the basketlike nuclei, and scattering of Nissl substance.

LOCAL TREATMENTS

In order to obtain further information concerning the nature of the shock reaction involved, treatments were applied locally to the testes and general examinations carried out. For this work the treatment distance was shortened, making the intensity higher and the treatment period less than 30 minutes. The entire animal was shielded with lead except the testes which were drawn out from the body with thread and soft string in such a manner as not to impede circulation. Figure 4, *A*, shows a section of a testis which was fixed 2 hours after the beginning of a 25,000-r treatment. Extensive cell degeneration and cell loss occurred even during this short interval. Figure 4, *B*, shows how extensive this became in 36 hours.

Figure 5 shows sections of lungs taken from rabbits at 18 (*A*) and 36 hours (*B*), after a 25,000-r treatment applied locally to the testis. Partial atelectasis and some congestion were present at 18 hours. Within the next 24 to 48 hours these changes became extreme, and the lung structure itself began to undergo necrosis. The changes which took place between 18 and 36 hours were probably not so marked as indicated in figure 5, as such effects in the lungs were found to vary considerably from one animal to another. The point of importance, however, is the fact that conditions as extreme as those shown in figure 5, *B*, could be found within 36 hours.

EFFECTS OBTAINED WITH STILL HIGHER INTENSITIES

By bringing the two X-ray tubes as close together as possible, intensities of 7,000 to 10,000 r per minute were obtained over a small area. Mice placed one at a time in this field died after 22 minutes' exposure. Mouse testes treated locally with 50,000 r in 7 minutes and fixed at once showed shrunken hyperchromatic nuclei with some cytoplasmic retraction but no cytoplasmic or nuclear degeneration. It is, therefore, apparent that whereas a cell may be fatally injured in a few minutes, a somewhat longer time is required for cell disintegration to occur.

DISCUSSION

By raising the amount of irradiation to such heights that the animal died either during treatment or shortly thereafter, extensive tissue destruction was produced and a distinctive type of fatal shock was revealed. The lack of hemoconcentration and the suddenness of death observed (in the acute cases) sets this shock reaction apart from that usually seen following trauma, burns, and other types of injury. The deluge of cell-disintegration products in all probability would have provoked the usual shock syndrome if the host organism had lived longer and had been capable of reacting. The symptoms of hyperthermia and hyperesthesia with intermittent seizures and marked cyanosis suggest that the control centers were seriously damaged, allowing various delicately balanced physiologic systems to deviate seriously beyond the usual normal limits. These changes, combined with the insufficient respiratory function and generalized toxemia, were probably responsible for the early deaths.

Moon (*8*) has discussed the problem of irradiation sickness and has pointed out that the symptoms are typical of the usual form of shock: nausea, vomiting, oliguria, bloody diarrhea, restlessness,

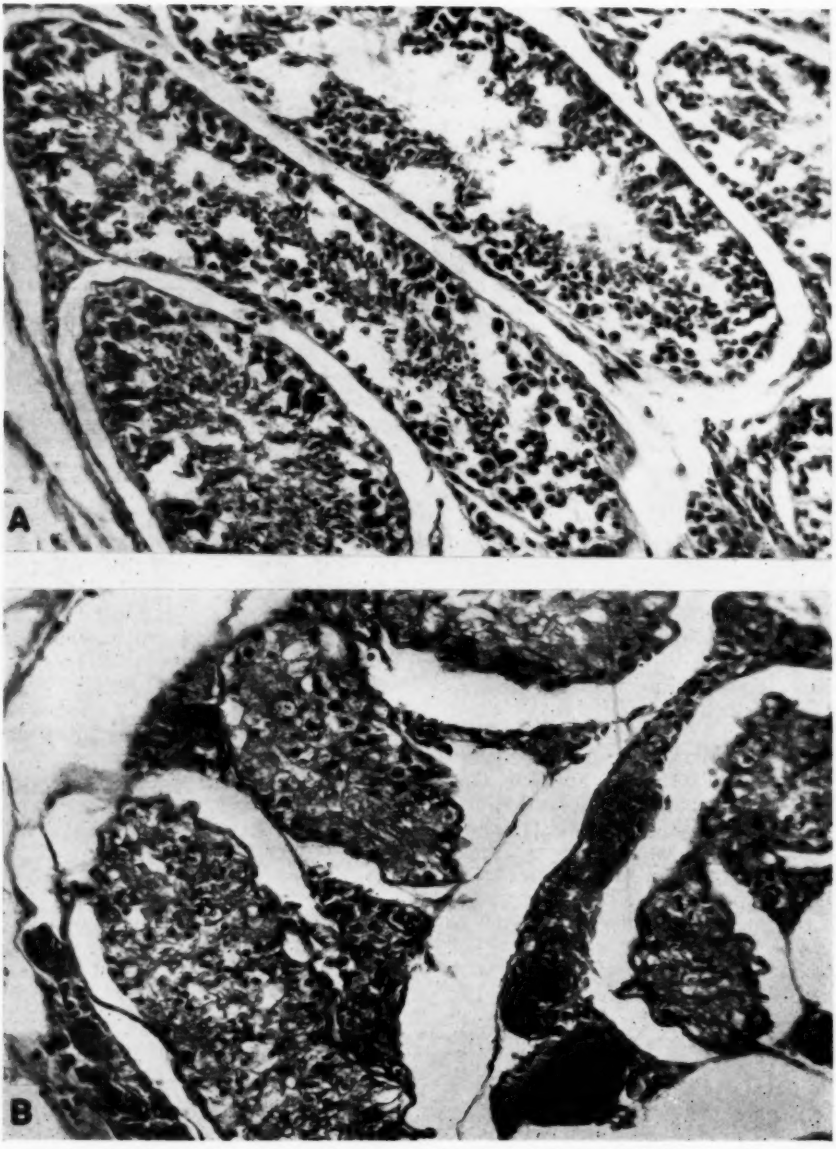

FIGURE 4.—A, Biopsy from the testis of a rabbit which had received 25,000 r locally to the scrotum; specimen taken 2 hours after the treatment began; *B*, Opposite testis taken from the same animal, 36 hours later. × 260. Hematoxylin-and-eosin stain.

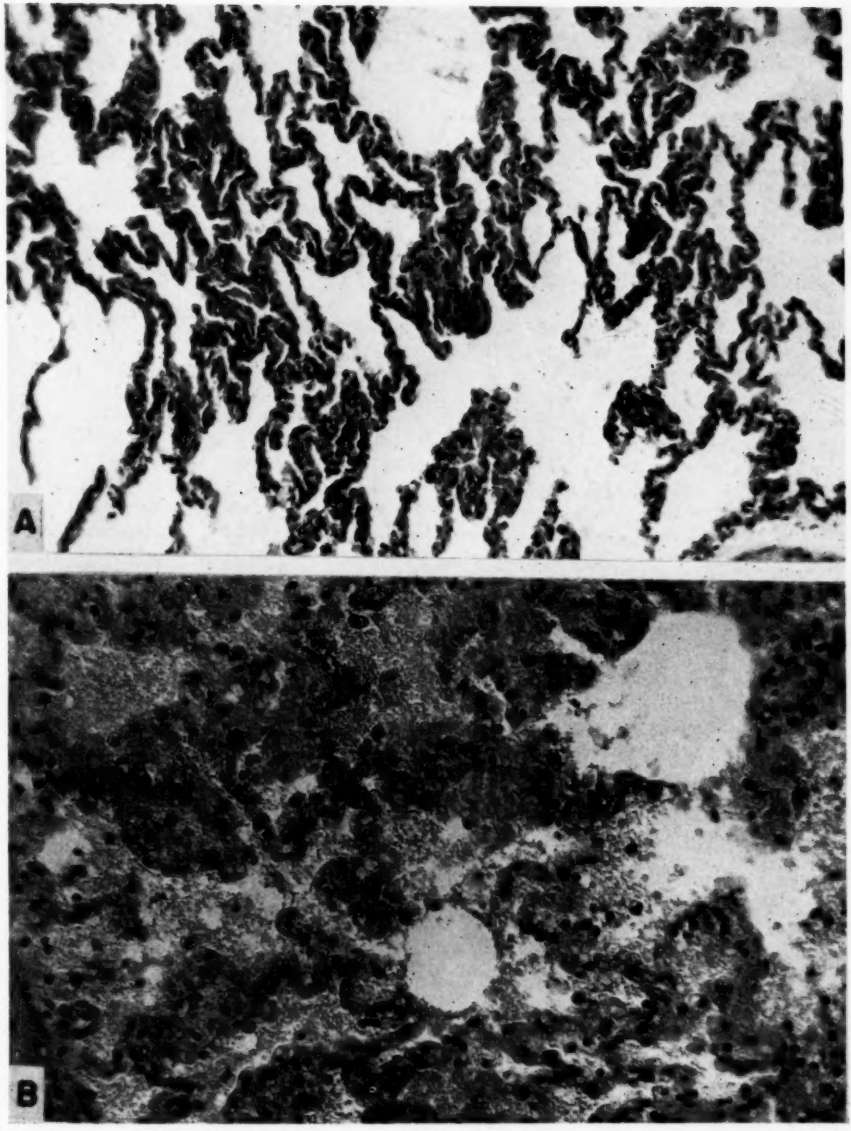

FIGURE 5.—*A*, Lung section taken from a rabbit 18 hours after local application of 25,000 r to the testes. × 100; *B*, Lung section from a rabbit treated in the same manner but killed at 36 hours. × 260. Hematoxylin-and-eosin stain.

small rapid pulse, declining blood pressure, and profound prostration. This author administered 1,400 to 2,800 r locally to the abdomens of dogs and studied the changes which followed. No effects were observed for 24 to 60 hours after which symptoms began to develop. These were loss of appetite, vomiting, diarrhea, and a decrease in the output of urine. Traces of blood were seen in the urine, feces, and vomitus. These changes progressed steadily, and death from circulatory failure occurred usually within 24 hours after the onset of illness, that is, 3 to 5 days after the treatment. No changes in the erythrocytes and hemoglobin content of the blood were noted until the onset of symptoms. At this point, hemoconcentration appeared regularly and progressed commensurately with the severity of the symptoms. The hemoconcentration ranged from 15 to 30 percent. Post-mortem examination showed diffuse hyperemia of the viscera, and the lungs were mottled and cyanotic. Microscopic examinations showed capillo-venous congestion, and numerous capillary hemorrhages. The serous surfaces and the gastrointestinal mucosa were deeply congested. Bloody fluid was found in the intestinal lumen, and the lining had the appearance of purple velvet. The evidence of damage was most extensive in the mucosa of the small bowel. Microscopic examinations showed varying degrees of degeneration and necrosis of the epithelium and marked hyperemia of the capillaries and venules. Moon refers to irradiation sickness as a toxic reaction due chiefly to products of tissue disintegration. The descriptions given in this paper, as well as those in the one just preceding, show how extensive and how sudden this disintegration can be.

SUMMARY

The application of 50,000-r doses of X-rays to the whole bodies of guinea pigs, rabbits, and mice was followed by death in a few hours. Guinea pigs were dead at the end of treatment (3 hours), rabbits within 3 to 6 hours after treatment, and the mice within 24 to 48 hours.

Death appeared to result from a distinctive type of shock, symptoms of which were generalized toxemia, hyperthermia, hyperesthesia with intermittent seizures, and cyanosis. Hemoconcentration was not seen in the acute cases.

Widespread tissue destruction occurred during these short intervals. Tissue sections showed extensive nuclear and cytoplasmic degeneration followed by extensive parenchymatous cell loss.

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EXPERIMENTAL ROENTGEN INJURY. IV. EFFECTS OF REPEATED SMALL DOSES OF X-RAYS ON BLOOD PICTURE, TISSUE MORPHOLOGY, AND LIFE SPAN IN MICE

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The changes induced by repeated small doses of X-rays is of interest because of various physiologic complications or diseases late in life which may unsuspectingly result from exposure to ionizing radiations.

Workers in medicine, industry, and science have sometimes been occupationally exposed to doses amounting to several r per day. Hence, in planning work with animals, it was decided to simulate the conditions of occupational exposure. Since this study was undertaken before the findings of the three previous papers (*1*, *2*, *3*) were available, the writer was not aware that detectable tissue injury could be induced by single acute doses of 50 r, or that recovery from relatively mild injury in some cases required as much as several weeks. In selecting the doses, use was made of the fact that the accepted "tolerance" or "permissible" dose for human beings is set at 0.1 r for 24 hours (*4*), and that this is considered to have a generous margin of safety (*5*). Thus, since there seemed little likelihood of obtaining clear-cut changes with doses less than 5 r per day, this amount was chosen as the lowest daily dose.

MATERIAL AND METHODS

C3H male mice 2 to 3 months of age when treatment started were used exclusively. Treatments were given daily five times per week. This schedule was used in order to reproduce more nearly the work week. Daily doses of 5, 10, 15, 20, and 25 r were administered until the animals died. The intensity of radiation used was 8.0 r per minute as measured in air with a Victoreen ionization instrument. Hence the treatment times were 0.62, 1.25, 1.87, 2.50, and 3.13 minutes, respectively. The X-ray treatment conditions were 200 kv., 20 ma., 0.5 mm. copper and 1.06 mm. aluminum filter, and the distance from the center of the target of the X-ray tube to the center of the animals was 105.3 cm. Only a single X-ray beam was employed as only small doses were required. Actual exposures were carried out by placing the animals in a shallow celluloid tray in the X-ray beam as described previously (*1*). Ten animals were used for each experimental dosage and also for controls.

Two complete experiments were performed, the second being a duplicate of the first, except for one feature. In the first experiment, since it was partly exploratory in character, treatments were discontinued on half of the animals after 12 weeks. This report is based upon 60 animals for each experiment, or a total of 120.

At intervals of 2 weeks, total leukocyte and differential counts were made on the blood of two animals of each exposure group and controls, the same two animals being used each time. Counts as well as treatments continued until the animals died (i. e., with the exception of half of the animals in experiment 1). At death, autopsy was performed, and tissues were fixed for histologic study. Thus, a continuous record of the blood picture, a description of the gross changes (at death), a histologic tissue series (also at death), and a record of the length of life were available for study.

RESULTS

Figure 1 shows curves of total leukocyte counts for a period of 24 weeks (approximately 6 months). These curves are based on the averages for two animals in each case. Curves for the animals receiving 10 and 20 r per treatment were omitted to avoid crowding. Zero on the abscissa indicates the time when treatment started. The point

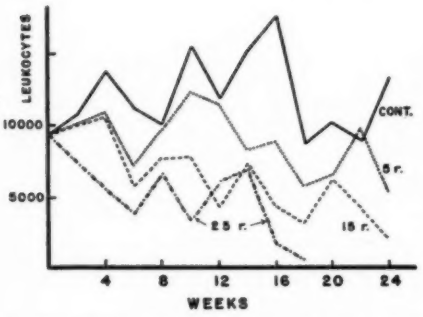

FIGURE 1.—Total leukocyte counts over a period of 24 weeks for animals receiving 0 (controls), 5, 15, and 25 r per treatment as indicated.

plotted at zero time represents the control value obtained by taking the average of counts on two animals picked at random just before treatment started. The precount value, which was 9,275 (leukocytes per cubic millimeter of blood), furnishes a starting point for all curves. Two counts were used here to give the control value the same weight as the experimental.

The solid-line curve (fig. 1) shows that the range of variation in nonirradiated control animals was from 8,800 to 17,725 (the extremes for the individual animals were only slightly different, being 7,100 to 19,800). The average for the control series was 12,300, which is higher than the initial precount value but nearer to the average of 11,500 previously obtained for 39 animals of the same species (*1*). The dotted-line curves show that the leukocyte level for experimental animals dropped off progressively with time, and that the amount of fall varied directly with the size of the daily dose. Points for the 10- and 20-r data, when plotted, fall among the other points properly with respect to dosage.

Figure 2 shows the results of differential counts. In the graph for neutrophils, the curves for animals receiving 5 and 15 r per day follow the same general course as that for the controls. Likewise the curves for animals receiving 10 and 20 r per day, when plotted, run generally similar to those for the controls. The curve for animals receiving 25 r per day, on the other hand, is generally lower. In the graph for lymphocytes, even the curve for animals receiving 5 r per day falls significantly below that for the controls, the others appearing progressively lower in accord with dosage. In the case of the higher dosages, a low of 1,000 to 2,000 cells was reached in 4 to 8 weeks. A comparison of the graphs for neutrophils and lymphocytes reveals not only that the lymphocytes were more readily affected than the neutrophils but also that the lymphocyte change became manifested at an earlier time.

The curves (figs. 1 and 2) cover a period of 24 weeks. However, the animals in most cases lived longer than 24 weeks, and blood records were kept. The curves have not been extended because the boed picture in the experimental animals became very erratic, and graphing the data only confused the picture. The leukocyte counts, irrespective of irradiation doses, swung from lows of a few hundred to highs of 20,000 or more. One animal receiving 5 r per treatment showed a count of 29,000 4½

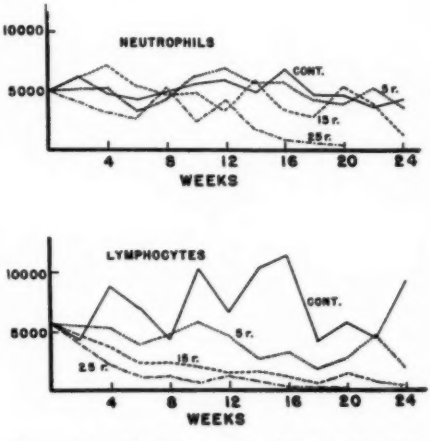

FIGURE 2.—Absolute neutrophil and lymphocyte numbers. The values plotted were obtained by making percentage differential counts and calculating the actual cell number from the total leukocytes.

months before death. Six weeks later, the count was 1,500. After 6 weeks, it varied from 8,000 to 13,000 until just before death, when it dropped to 1,800. In an animal getting 10 r per treatment, after varying between 3,000 and 8,000 for several months, the count suddenly shot up to 34,000 just before death. Both animals that received 20 r per treatment showed oscillations in the terminal stages from 3,000 to 15,000. In the case of the animals that received 25 r per treatment, the low counts were lower and the upswings were not so great.

GROSS PATHOLOGY

The gross pathologic changes were primarily those of aging: excessive graying of the hair, emaciation, marked loss of body fat, and generalized atrophy, the last-mentioned being most noticeable in the lymph nodes, spleen, and testes. In those animals which had received higher daily doses, the testes were little more than small brown flaccid bags, and the lymph nodes and spleen were very small. In some of the animals ulcers were found in the lower ileum. A few animals in the groups receiving 15 and 20 r per treatment showed generalized lymph-node enlargement, enlarged spleens, and oversized livers which were grayish red.

HISTOPATHOLOGY

Since tissues were taken at autopsy after natural death, the histologic study dealt only with terminal conditions. It might have been presumed, therefore, that death occurred when the same degree of tissue injury had accumulated in each animal, irrespective of the daily dose. One of the strongest impressions gained from the study of the histologic material, however, was that the amount of tissue injury varied with the different daily treatments.

LYMPH NODES AND SPLEEN

Figure 3 shows typical sections of lymph-node material from animals receiving different daily doses. The photomicrographs fail, of course, to show the range of the variability in each dosage group, but the conditions illustrated are considered as representative. The areas chosen show part of the capsule opposite the hilus in each case. All irradiated lymph nodes showed evidence of injury. However, if material from the different groups is compared, wide differences are seen, varying from an unhealthy hyperplasia in the 5-r material to an extensive aplasia in the 25-r material. Many small lymphocytes were present in the 5-r samples (and controls), whereas only swollen reticular cells and fibrous connective tissues were found in the material receiving the heaviest daily treatments. The lymph nodes of animals receiving doses of 10, 15, and 20 r per day showed intermediate stages. The conditions differed irregularly in each group of animals but tended to vary progressively with the amount of daily treatment. In connection with the irregularity displayed here, it should be recalled that the peripheral blood picture showed large fluctuations for a period of a few days or weeks just prior to death. Still other conditions of hyperplasia were observed in the intermediate-range group, but these are described later.

Changes in the spleen were similar to those in the lymph nodes although usually not so severe. Occasionally, this organ showed concentrated accumulations of megakaryocytes, but this was not a constant finding.

BONE MARROW

Bone marrow of the sternum and vertebrae were studied. Figure 4 shows sections of marrow from the various dosage groups. As in the case of the lymph nodes, the degree of injury tended to vary directly with the size of the daily dose. The marrow of animals receiving 5 r per treatment was for the most part hyperplastic, although there were occasional sinusoidal spaces containing no marrow substance. The ratio of cell types appeared normal except that there was a greater proportion of more adult forms. The 25-r material on the other hand was extremely hypocellular.

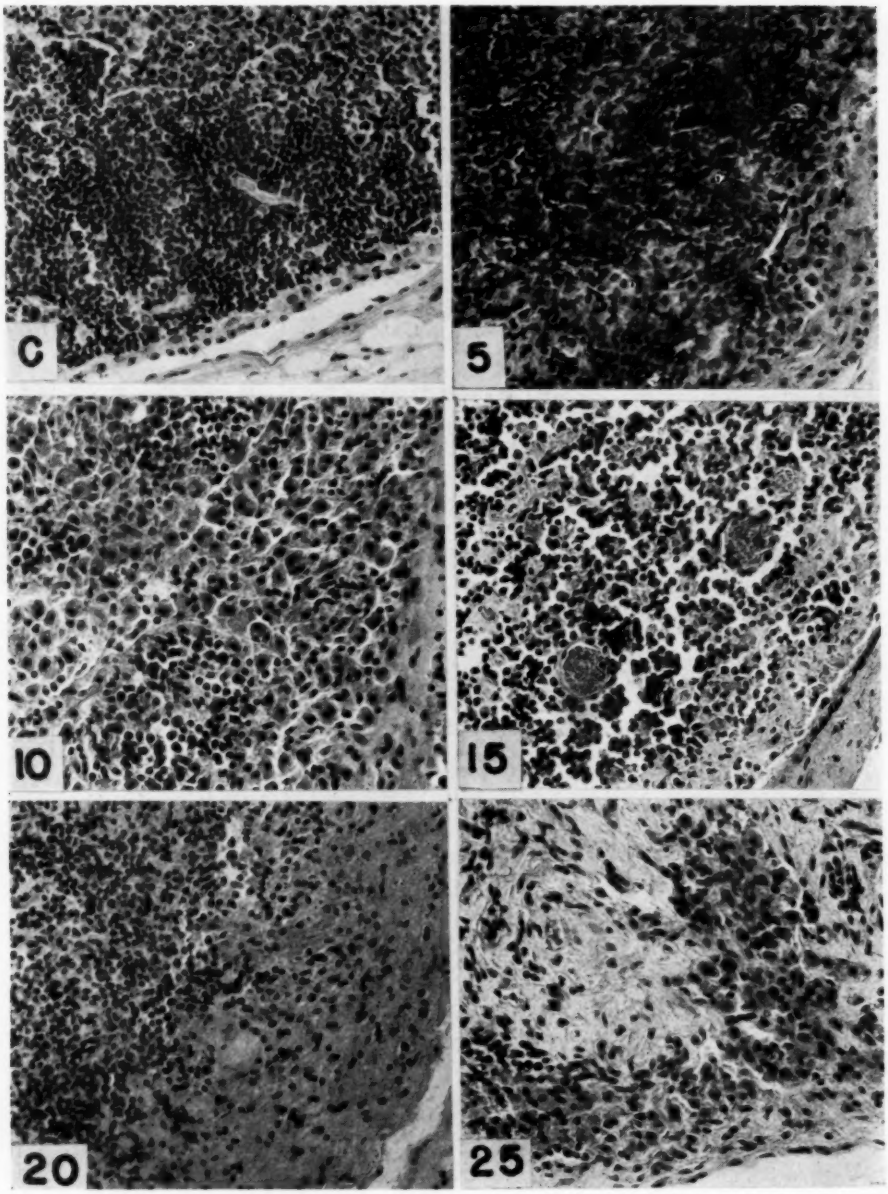

FIGURE 3.—Lymph nodes arranged in accordance with the size of daily dose as indicated. *C* indicates nonirradiated control material. × 235.

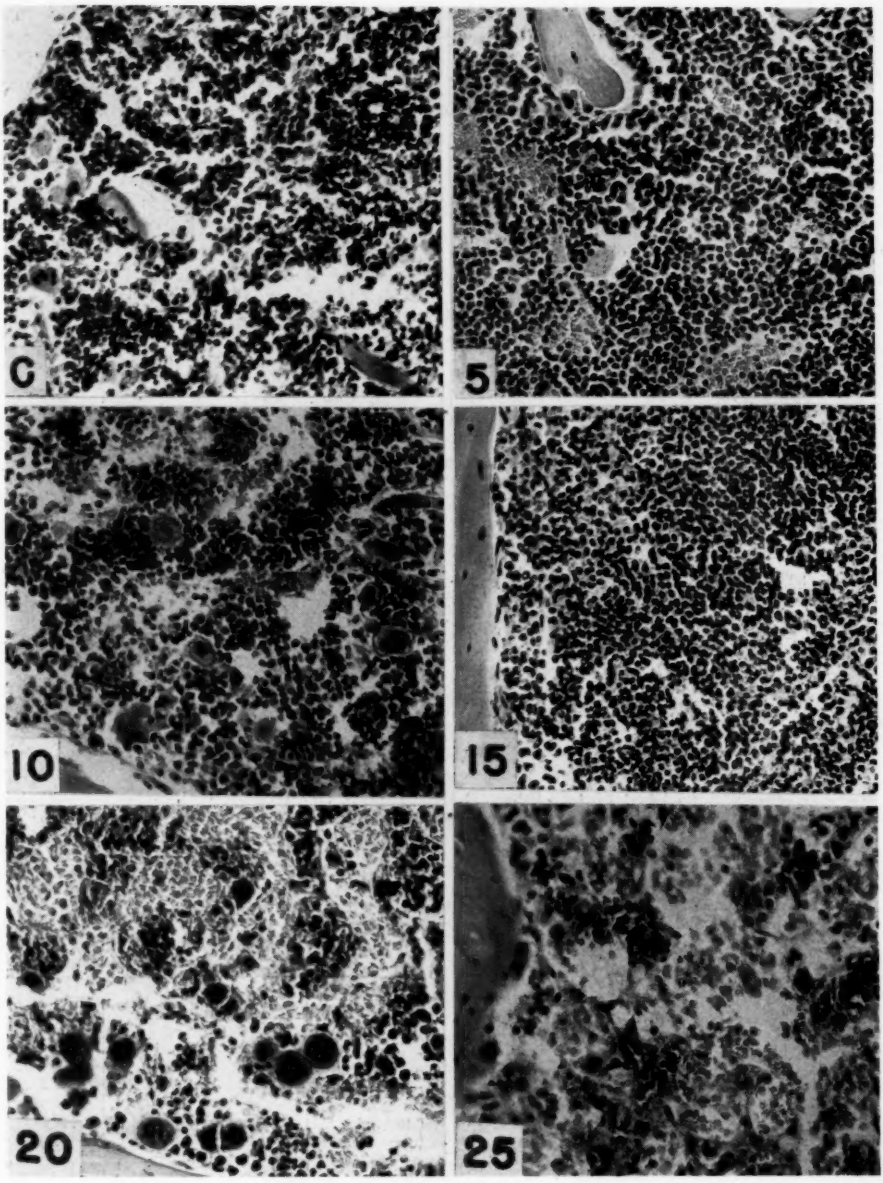

FIGURE 4.—Bone-marrow sections arranged in accordance with the size of the daily dose as indicated. × 235.

Marked acellularity was uniform in this dosage group; some animals showed even fewer cells than pictured in figure 4, 25. The marrow of mice receiving 15 and 20 r was in most cases similar to that of animals that had received 25 r, showing extensive aplasia. Occasionally, material was found which resembled that from mice given doses of 15 r; this showed hyperplasia and many cells with ring-shaped nuclei. The marrow of animals receiving 10 r showed both aplasia and hyperplasia.

In addition to the foregoing findings, a few animals (particularly those in the first experiment receiving 15 and 20 r per treatment for 12 weeks only) showed a hyperplastic marrow with a fairly large primitive-type cell (with vesicular, round, or kidney-shaped nucleus) as the predominant type. This cell was prominent not only in the marrow but also in the hematopoietic organs, and surrounding musculature; large numbers were present in the kidneys, liver, lungs, and other organs.

TESTIS

Figure 5 shows seminiferous tubules from animals receiving the different daily doses. Again the photomicrographs fail to show the range of variability but are considered representative. Figure 5, *C*, shows tubules of a nontreated control animal that had died a natural death at 13 months of age. In these tubules all germinal elements were present, and active spermatogenesis appears to have been in progress. As emphasized previously (*1*), the degree of activity may vary in different tubules, some showing few secondary spermatocytes (as at the top of figure 5, *C*). In no case, however, has an untreated testis been found to contain tubules with a complete absence of primitive germinal elements.

All irradiated testes showed extensive injury. In these organs there was no hypercellularity, only loss of cells, the degree of which varied in a general way with the size of the daily dose. In the tubules of animals receiving 5 r per treatment, many primary spermatocytes were present although in badly damaged condition, while in those receiving 25 r few or no spermatocytes were present. The tubules contained sertoli cells almost exclusively. The 25-r material was uniformly acellular; some animals had even fewer sertoli cells than shown by figure 5, 25. The 5-r material also displayed variability, some animals having somewhat less injury than shown, others more. The testes of animals receiving 10, 15, and 20 showed variations between the extremes of 5- and 25-r material, but the tendency was toward an absence of germinal elements as indicated.

LEUKEMIA

It has been indicated already that some animals at death showed an extensive primitive-cell invasion of various organs. Because of the character of this cell and its invasive behavior, the condition which developed has been regarded as a form of leukemia. Since persisting high levels of leukocytes were not maintained in the peripheral blood, the type has been considered pseudoleukemia or aleukemic leukemia. Table 1 shows the distribution of leukemia cases among the various dosage groups. It was mentioned previously that half of the animals in experiment 1 received treatment for 12 weeks only; most of the leukemic animals appeared in this group. Further, most of the leukemia cases occurred in animals that received treatment of 15 and 20 r per day.

TABLE 1.—*C3H male mice showing leukemia after treatment with various doses of X-rays*

Daily treatment (in r)	Experiment 1		Experiment 2
	21 weeks' treatment only [1]	Continuous treatment [1]	Continuous treatment [2]
	Number	*Number*	*Number*
0 (controls)	0	0	0
5	0	0	0
10	1	0	0
15	3	0	2
20	3	1	0
25	1	0	0

[1] 5 animals per sample.
[2] 10 animals per sample.

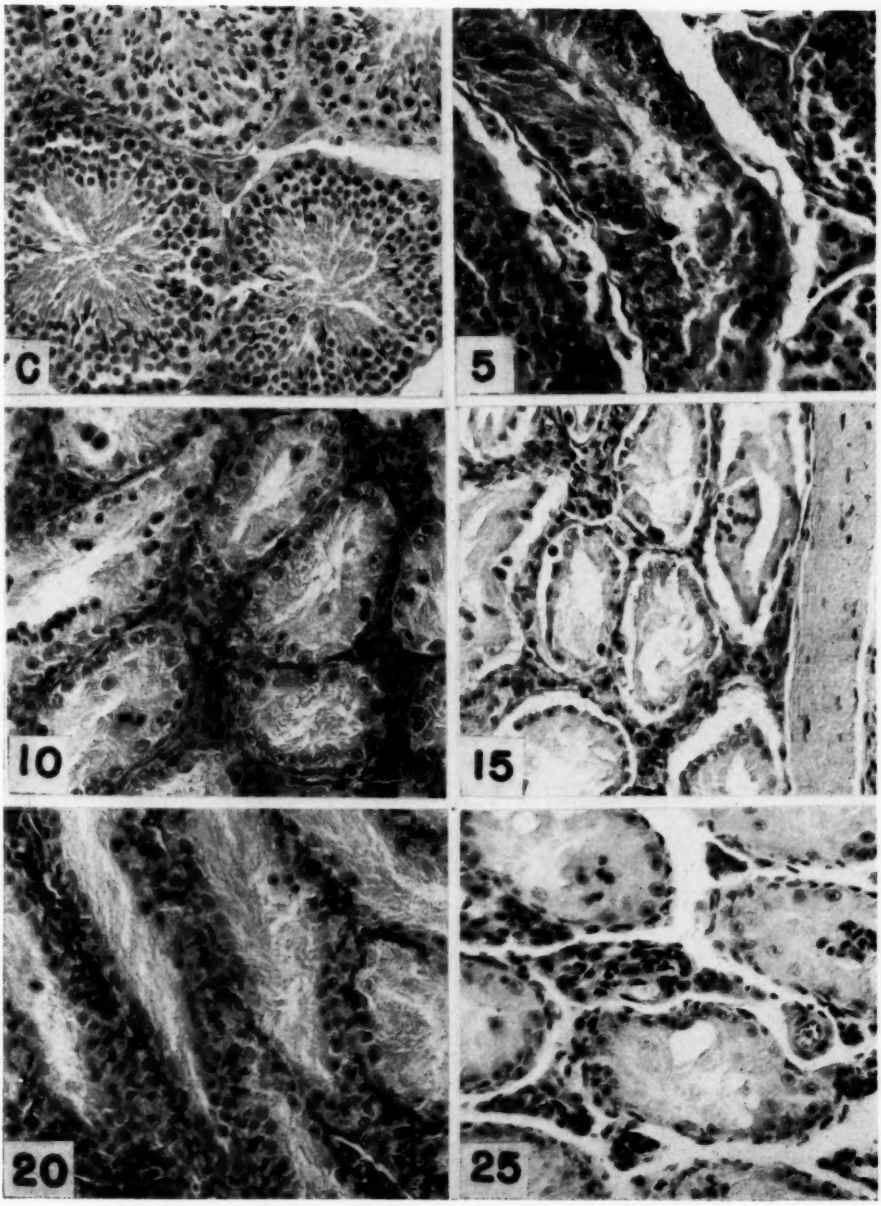

FIGURE 5.—Seminiferous tubules arranged in accordance with the size of the daily dose. × 210.

DURATION OF LIFE

The length of life of each animal (after treatment began) was obtained from the treatment and death records. The life span in months for individual animals is shown in table 2. Since part of the animals in experiment 1 were exposed for 12 weeks only, their survival periods were not comparable with the others and accordingly were not included in table 2. It will be noted from the data that considerable variation in time of death occurred among the animals of each experimental group. Accordingly the data were tested in a variety of ways. Separate curves were plotted for each experiment and the individual groups of data were studied. By every consideration the same general trend was found. Hence it seemed appropriate to combine the results of experiments 1 and 2. Figure 6 shows combined averages based on 5 animals for each experimental dosage for experiment 1 and on 10 animals in experiment 2. All points in this graph fit close to a straight line which extends downward to the right. The course of the curve here is considered as significant but the slope, because of the variability of the data, is regarded as only approximate.

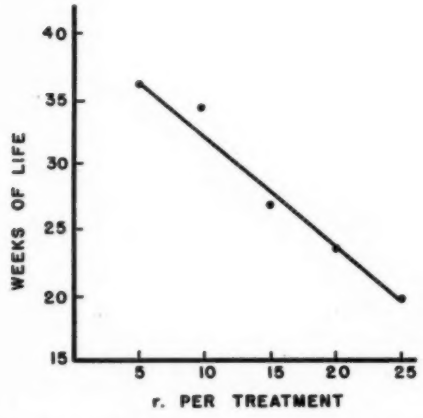

FIGURE 6.—Average length of life as a function of the daily dose.

The downward course of the curve (fig. 6) shows that the length of life varied inversely with the size of the daily dose of radiation administered. In other words, the larger the daily dose the shorter the span of life. The very regular distribution of points indicates that the length of life varied in a consistent manner with the amount of daily treatment. It is apparent from the table that the average life of the controls was more than 40 weeks; hence, since the average life of animals receiving 5 r per day was approximately 35 weeks, it is clear that the lowest daily dosage used was sufficient to cause some shortening of life. By extrapolating to the left, it would appear that the curve must turn upward (perhaps abruptly) to reach the average life of the controls.

When this curve is extrapolated to the right, it intercepts the base line (for zero length of life) between 45 and 50 r per daily dose.[1] This suggested that daily exposures of 45 to 50 r should produce death in a short time (at least a few days or weeks). A side experiment was carried out to test this possibility. Three groups of ten animals each were given 40, 60, and 80 r per day 5 times per week as in experiments 1 and 2. The average time of death was 11, 7, and 4 weeks, respectively. Thus, while the results do not show really sudden death after doses of 45 to 50 r, they do nevertheless indicate that the course of the reaction remains reasonably constant over a wide range.

ACCUMULATED DOSES

The average accumulated doses of radiation administered to each group of animals were calculated by determining the product of the number of daily treatments and the size of the daily dose. The values so obtained are shown in table 2, and points representing these values are plotted in figure 7 as a function of the daily dose. The curves as drawn show that the total dose varied with the sizes of the daily treatments.

[1] The base line of the graph is at 15 weeks of life rather than at 0.

TABLE 2.—*Length of life after treatment started and total dosage received by mice given various doses of X-rays.*

Dose (in r)	Mouse No. —										Average	Total dose
	1	2	3	4	5	6	7	8	9	10		
	Wks.	*Wks.*	*Wks.*	*Wks.*	*Wks.*	*Wks.*	*Wks.*	*Wks.*	*Wks.*	*Wks.*	*Wks.*	*r*
Experiment 1:												
0 (Controls)	[1] 44	[2] 10	35	[1] 44	[1] 44						42	
5	36	33	31	30	24						31	775
10	31	27	30	29	23						28	1,400
15	29	25	26	24	28						26	1,950
20	22	23	21	[2] 4	27						23	2,300
25	20	21	19	18	([2])						20	2,500
Experiment 2:												
0 (Controls)	40	43	41	39	50	41	41	62	62	39	45.8	
5	56	44	34	53	34	31	41	35	35	35	39.8	995
10	38	37	36	39	37	38	39	38	39	38	37.9	1,895
15	27	24	32	33	29	25	30	24	31	20	27.5	2,063
20	23	24	24	23	28	24	24	24	29	18	24.1	2,410
25	20	18	25	17	17	21	16	19	20	18	19.1	2,386

[1] Mice killed.

[2] Not used in average.

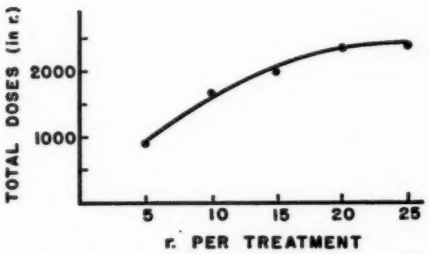

FIGURE 7.—Total accumulative doses for different daily treatments.

The fact that the curve progressed upward as the size of the daily dose was increased indicates that the larger the daily dose, the larger the total dose acquired before death. This was true, despite the fact that animals receiving smaller daily doses actually lived longer (fig. 6).

DISCUSSION

Different-sized daily doses of X-rays were administered to mice continuously until death, and various progressive and terminal modifications were observed. These consisted of (1) a fall in leukocyte level, (2) marked and erratic blood fluctuations shortly before death, (3) extensive tissue injury, (4) atypical leukemias, and (5) a shortening of the life span. The fall in leukocyte level, the degree of tissue injury, and the shortening of life were all found to vary directly with the size of the daily dose. The size of the total accumulated doses given to the different experimental groups, on the other hand, was found to vary inversely with the size of the daily dose.

The shortening of life owing to intermittent small exposures is an effect not systematically studied heretofore, as far as is known. Since it is plain that the course of the reaction extends over a wide range of exposures and since it is also clear that the mice receiving 5 r daily did not live as long as the controls, the question is raised whether 2.5, 1, or even 0.1 r per day might not affect the length of life, especially in organisms the life span of which is appreciably longer than that of the mouse. This question is of particular interest in the case of human beings who may be exposed to small amounts of X-rays or gamma rays daily over a period of many years. The data for the mouse indicate that 5 r per day is not far below the threshold of injury so far as length of life is concerned, but the results of the experiments are too unstable to permit a reliable extrapolation.

The curve (fig. 7) shows a rather surprising feature. It extends upward to the right, apparently approaching a peak or plateau in the neighborhood of 25 to 30 r per daily treatment. The fact that this curve extends downward to the left, that is, in the direction of smaller daily doses, means that although the animals receiving smaller daily doses

lived longer, they actually received less total radiation before death. The course of the reaction, therefore, was different from many of this general type. In most balanced biologic systems, some degree of recovery usually follows a shift from the normal condition caused by some agent. In such cases, the treatments are not entirely additive if significant periods of time are allowed for recovery; larger doses are required to produce the same degree of effect when the treatments are fractionated.

In the present experiment this general picture was reversed, as actually less total radiation was applied in the case of the smaller daily doses where the recovery should have been more complete. Here the daily dose not only failed to be fully additive but also the velocity of the reaction appeared to increase with an increase in the amount of daily treatment. Just why the lethal dose of radiation should have been less when the daily dose was smaller has not been ascertained although an explanation is suggested by the combined experimental and histologic findings. It will be recalled that the over-all picture of the effect of radiation on the tissues was one of aging (loss of parenchymatous cells together with poor regenerative ability). If radiation acts to hasten the normal process of aging, normal aging would be expected to exert a relatively greater effect in those animals that lived longest, as was observed.

Finally, it is clear that the daily doses used did not reveal the threshold dose for any of the effects studied. However, by examining the curves (figs. 1 and 2), it seems doubtful whether effects produced in the peripheral blood by doses of less than 5 r per day could be distinguished from the condition in controls. On the other hand, histologic damage was pronounced in hematopoietic organs and testis and the life span was appreciably shortened by daily doses of 5 r. Hence, it seems likely that some tissue injury would be detected and the length of life decreased with still lower doses.

SUMMARY

Small daily doses of 5, 10, 15, 20, and 25 r per day were applied to the whole bodies of C3H mice.

There was a fall in leukocyte level, owing mainly to lymphocyte loss. The amount of fall varied progressively as treatments proceeded and directly with the size of the daily dose.

Extensive parenchymatous tissue damage was seen in the lymph nodes, spleen, bone marrow, and testis, the degree of which varied in a general way with the size of the daily dose.

A shortening of life span was noted, the amount of which likewise varied directly with the daily dose.

Leukemia developed in some of the mice receiving 15 and 20 r daily.

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MINIMAL NUMBER OF ANESTHETIC TREATMENTS WITH URETHANE REQUIRED TO INDUCE PULMONARY TUMORS

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In a previous communication [1] it was shown that a marked increase in incidence of lung tumors in C3H mice followed the weekly administration of anesthetic doses of urethane (ethyl carbamate). The incidence rose from less than 5 to more than 80 percent. Such increases were obtained with different lots of the drug as well as with a purified sample, and tumors were seen as early as 2 to 3 months after the beginning of treatment. Tumors were obtained irrespective of whether the urethane was given in anesthetizing doses or in fractionated subanesthetizing doses. Exploratory experiments indicated that only a few, probably only 1 or 2, anesthetizing doses were required for tumor induction. In strain A mice which ordinarily show spontaneous lung tumors in about 75 percent of the animals at 18 months of age,[2] 45 to 64 peripheral tumors were found in animals 6½ months of age that had received 14 weekly anesthetic treatments. Only 1 animal in a similar set of strain A controls showed a single peripheral tumor.

The purpose of the present work was to determine in more precise manner the minimal number of anesthetic doses required to induce pulmonary tumors, and something of the relative effects of different numbers of treatments.

In order to hasten the work, strain A mice were used exclusively. Intraperitoneal injections of a 10-percent aqueous solution of urethane (1 ml. per 100 gm. of mouse) were started when the animals were 6 to 8 weeks old. Six groups of twenty animals each were used. The first was the control group, and it received no injections. The second group received a single injection, and the remaining groups received two, three, four, and five injections, respectively, an interval of 1 week occurring between each treatment. At 4½ months after the first treatments were given, that is, when the animals were 6 to 6½ months old, all were killed and examined for the presence of lung tumors.

At autopsy, the lungs were removed and dropped into Tellyesniczky's fluid. This fluid causes the surface tumors to stand out as white, elevated nodules in contrast with the normal lung tissue. These nodules were counted with the unaided eye, and the number (peripheral index) was recorded. From our previous experience where serial sections (every tenth section being saved) were made of the lungs, we felt competent to distinguish the tumors with a high degree of certainty. While the number of tumors seen undoubtedly would have been increased by the use of serial sections and magnification, the peripheral index was considered adequate for purposes of comparison.

Table 1 gives the peripheral index of lung tumors seen in each animal. This compilation shows that whereas only a single tumor was seen in the control animals, 6 to 20 were found in each animal of the series receiving a single treatment and that even more tumors were seen in the animals receiving additional treatments. The averages show a progressive increase in number of peripheral tumors with number of treatments up to and including 4. Points for the average values have been plotted in figure 1, which shows that the rate of increase is remarkably uniform.

Just why the number of tumors obtained after 5 treatments should be less than that obtained after 4 is not clear. However, from the fact that 45 to 64 tumors were obtained with 14 weekly treatments 4½ months after the begin-

[1] NETTLESHIP, A., and HENSHAW, P. S.: The induction of lung tumors in mice with ethyl carbamate (urethane). J. Nat. Cancer Inst., 4: 309–319 (1943).

[2] BITTNER, J. J.: Breast and lung carcinoma in "A" stock mice. Pub. Health Rep., 54: 380–392 (1939).

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ning of treatment (*1*), it is clear that the maximum effect is not reached with 4 treatments. The previous and present results taken together likewise suggest that the uniform rate of increase indicated by the 1-, 2-, 3-, and 4-dose treatments (fig. 1) does not extend indefinitely, as extrapolation of the straight line section shows that over 100 tumors would be expected for 14 treatments. Considering the fact that the later treatments had only a short period of time during which their influence could become manifested, it seems at least probable that this straight-line relationship might be maintained over a considerable range if the treatments and growth intervals could be properly managed.

TABLE 1.—*Lung tumors per animal*

Animal No.	Number of anesthetic treatments					
	0	1	2	3	4	5
1	0	13	26	18	37	44
2	0	7	21	29	31	51
3	0	7	34	35	21	10
4	1	8	0	33	40	37
5	0	6	9	8	31	32
6	0	13	21	57	66	47
7	0	10	22	14	54	17
8	0	12	19	29	31	70
9	0	8	17	48	31	19
10	0	8	22	28	46	21
11	0	7	15	6	39	55
12	0	12	17	17	29	34
13	0	13	12	38	40	53
14	0	8	14	30	40	24
15	0	6	15	21	35	27
16	0	20	11	12	37	16
17	0	4	10	7	18	25
18	0	9	23	22	24	12
19	0	[1]	12	34	[1]	17
20	[1]	[1]	[1]	[1]	[1]	28
Average	.05	9.5	16.8	25.6	36.1	32.0

[1] Did not survive the duration of the experiment.

The data obtained give a complete answer to the question of the minimal number of anesthetic doses required to produce lung tumors. For the conditions used and for the material tested, it is clear that a single treatment is sufficient to induce an appreciable number of tumors in each animal. By interpolation (fig. 1) between 0 and 1 dose, it appears that some induction would probably be obtained by a single treatment involving less than the anesthetizing dose of urethane.

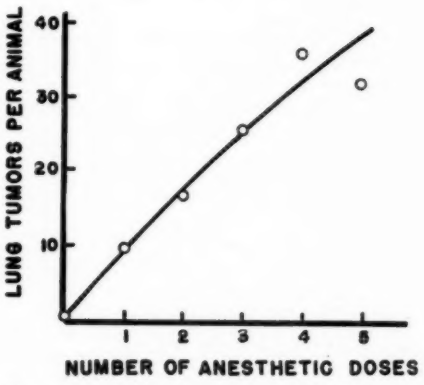

FIGURE 1.—Average number of lung tumors per animal plotted as a function of the number of anesthetic treatments administered.

Some of the interesting tumor problems are posed by this simple experiment. As stated in the introduction, a high percentage of the animals of this strain develop lung tumors spontaneously. However, only a few tumors are seen before the age of 1 year, and only rarely is one seen as early as 6 months. Likewise, it is rare, if ever, that tumors are observed to appear spontaneously in 100 percent of the animals at any age if groups of 100 animals or more are used. Thus, if we assume that the tumors which formed are in part those that would have arisen spontaneously at a later time, it is apparent that some tumors were formed which never would have arisen under other circumstances. It is not clear whether any of the tumors arose from the same cell constituency at this early stage as they would had they arisen later. If the precursor cells normally undergo mitosis, it is obvious that the cell of origin could not be the same in the two cases.

Just why certain cells respond to carcinogenic agents while their neighbors do not is not known. So far as we were able to tell there was no predilection as to site, the tumors appearing in all lobes and in all locations of the parenchymatous tissues. Occasionally two or more foci were close together, in some cases

appearing to touch and even coalesce. This suggests either that certain vulnerable cells or cell groups lie at random throughout the organ or that the agent accumulates to a greater extent at these points. We have observed that more treatment produces more foci, but this fact furnishes no evidence whether graded susceptibility of cells or varying concentration of agents is significant. In this connection, it is noteworthy [3] that no histologic evidence of lung-cell damage (either bronchial or alveolar) was seen to precede the formation of tumors. From this fact it would appear that the location of foci is not determined by areas of injury.

One animal in the two-treatment series (table 1) showed no tumors. Since all of those animals receiving only one treatment showed some tumors, it is remarkable that any animal receiving two treatments should have failed completely to manifest tumors of some kind. This observation suggests that some animals possess no tissue capable of responding to urethane by giving rise to tumors.

Perhaps one of the most significant observations was a negative finding not mentioned heretofore. In our experience with urethane thus far, other types of tumors, that is, tumors in organs apart from the lungs, have not been seen. Chemical carcinogens for the most part have not shown such selectivity. Methylcholanthrene, for example, injected subcutaneously or intraperitoneally, may induce tumors at the site of inoculation or remotely in the lungs, kidneys, bone marrow, or elsewhere. In the case of urethane, tumors have always developed at sites distant from the point of inoculation. These observations, together with the findings that little or no tissue injury precedes the appearance of lung tumors, make it plain that urethane carcinogenesis has characteristics usually associated only with viruses, specific tissue action, remoteness of induction site, and absence of precancerous injury.

Urethane is a water-soluble agent. It also has an important chemical relationship to the products of urea metabolism. These facts suggests new avenues of approach in problems of carcinogenesis.

In résumé, clear-cut evidence was obtained showing that a single anesthetic treatment is sufficient to induce pulmonary tumors in mice. By interpolation, evidence was obtained suggesting that some induction would probably be obtained by single treatments involving less than the anesthetic dose. The salient points derived from the urethane studies to date may be listed as follows: (1) Lung tumors, and apparently only lung tumors, are obtained remote from the site of injection; (2) the number of tumors obtained varies directly with the number of treatments; (3) the induction interval may be as short as 2 months; (4) tissue injury appears not to precede the formation of tumors; and (5) there appears to be no predilection for sites so far as lung parenchymatous tissue is concerned.

[3] See footnote 1.

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CHANGES IN THE ADRENAL GLANDS OF RATS FOLLOWING EXPOSURE TO LOWERED OXYGEN TENSION

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INTRODUCTION

The importance of the adrenal glands in adaptation to various forms of stress (*1*, *2*) is well-known, and several morphologic (*3–8*) and biochemical (*9*, *10*) studies have been made to correlate structural changes in the adrenals with the degree of activation under various conditions. Some of these studies have dealt with adrenal changes in relation to exposure to lowered oxygen tension, but in these instances emphasis was placed upon changes in adrenal weight and not upon changes in adrenal histology. Sundstroem and Michaels (*11*) described, among other phenomena, extensive pathologic modifications in the adrenal glands of rats exposed continuously to several levels of lowered barometric pressure. Their results, however, cannot be compared directly with those reported herein since our results were derived from experiments on relatively short and repeated exposures. Langley and Clarke (*12*) investigated the problem of the reaction of the adrenals to anoxia, but they were mainly concerned with the relationship between adrenal hypertrophy and changes in carbohydrate metabolism. Armstrong and Heim (*13*), working with rabbits, studied the effect of repeated exposure to anoxia and included a description of the histologic changes in the adrenals in their report. Thorn, Jones, et al. (*14*) studied the effect of repeated daily exposure on adrenal weight and histology of both rabbits and rats.

The present study deals with the changes occurring in the adrenal glands of rats subjected to lowered oxygen tension in single and repeated exposures of short duration and is a part of a more comprehensive program designed to study the changes in various organs under the same conditions. It was felt that the results of such a study might be of some value in interpreting the changes resulting from chronic or accidental exposure to anoxia in aviation.

MATERIALS AND METHODS

Male Sprague-Dawley rats were used in all experiments and were exposed to a pressure equivalent to 27,000 feet altitude (258.1 mm. Hg, pO_2 c. 54 mm. Hg) for 4 hours in the acute exposures and to 25,000 feet (282 mm. Hg, pO_2 c. 59 mm. Hg) for 4 hours 6 days a week for the repeated exposures. The animals were exposed in a large steel decompression chamber. During the exposures a ventilation rate of 708 l. (25 cubic feet) per minute measured at sea level was maintained. The temperature was maintained between 23° and 25° C. The pressure level equivalent to 27,000 feet was used in the acute experiments since it was found that under our conditions this level was just below the ceiling for this strain of rat. The level of 25,000 feet was selected for the repeated-exposure studies since previous experience indicated that normal healthy male rats of this strain would easily tolerate repeated exposure at this level for long periods.

In the single exposure, or acute series, all adrenal glands were fixed in Champy fluid (1 part 3 percent potassium dichromate, 1 part 1 percent chromic acid, and 1 part 2 percent osmic acid) immediately after removal from the animals under nembutal anesthesia. One gland of each pair was fixed whole, and the other was fixed after being sliced through to the medulla with a razor blade. This latter procedure was found necessary if satisfactory samples of inner reticularis and medullary tissue were to be obtained. In the repeated-exposure series wet weights of the glands were first determined on a Roller-Smith

balance, and the glands were then fixed in Champy fluid in the usual manner. The adrenal weights in the acute and repeated-exposure series, therefore, cannot be compared directly, although rough comparisons may be made by assuming an average increase of 7 mg. for each pair of adrenals as a result of their fixation in Champy fluid.[1] After fixation, the adrenals were washed in running tap water for 24 hours, weighed, and then placed in 2 percent osmic acid at 37° C. for 6 days. After a second washing in tap water, the adrenals were dehydrated in graded alcohols, cleared in aviation gasoline, and embedded in paraffin in a vacuum oven. Sections were cut at 4μ and cleared and mounted unstained in balsam.

Armour's adrenal cortical extract[2] was used in the treated group of animals. Subcutaneous injections of this material in 0.5-cc. doses were made. All photomicrographs were taken of sections of adrenal glands fixed in Champy fluid, followed by impregnation with osmic acid, and have a magnification of 940 ×.

[1] In a later experiment adrenal glands taken from over 50 animals showed an average increase in weight of 7 mg. following fixation in Champy fluid.
[2] Hereinafter referred to as ACE.

RESULTS

In the examination of the "osmic acid reducing" droplets of the adrenal cortex, emphasis was placed on the zona glomerulosa, the "lipid free zone" of the zona fasciculata and the zona fasciculata proper. Attempts were made in the early part of the study to determine roughly the extent of change in droplet content. It was found, however, that changes in the zona fasciculata were too variable in degree for an average of such observations to be of any significance. Hence, in comparison with glands from normal animals, notes were made on the direction rather than the extent of change in droplet content in the glands of exposed animals.

ACUTE EXPOSURE

Changes in the adrenal glands of rats subjected to acute exposure are summarized in table 1. As noted previously, the paired adrenal weights in this series were taken after fixation in Champy fluid, and therefore they averaged 7 mg. heavier than their true fresh wet weights. No significant change in adrenal weight was noted immediately after a single exposure of 4 hours at 27,000 feet either with or without ACE

TABLE 1.—*Acute exposure of rats to lowered oxygen tension*

Treatment	Exposure	Number of animals	Body weight		Adrenal weight		Adrenal weight per gram body weight	Lipid content change		
			Average	Range	Average	Range		Glomerulosa	Lipid-free zone	Fasciculata
			Grams	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*			
	Controls	17	216	170–249	36.9	30.0–43.7	0.171			
	1 x 27,000	10	234	208–270	37.9	29.4–46.3	.162	0	+	3 to 7 0 to −
	1 x 27,000 [1]	6	230	193–265	38.5	33.8–43.8	.167	0	++	−
	2 x 27,000	4	212	186–225	44.5	34.6–53.7	.210	0	1 to 3 + to ++	−
ACE	Controls	22	251	218–280	42.0	34.0–50.0	.167	0	14 to 8 0 to +	20 to 2 0 to −
ACE	1 x 27,000	6	240	207–259	37.2	33.6–40.8	.155	0	+	5 to 1 0 to −
ACE	1 x 27,000 [1]	7	242	222–262	46.7	36.7–52.0	.193	0	++	6 to 1 0 to −

[1] Animals killed 24 hours later.

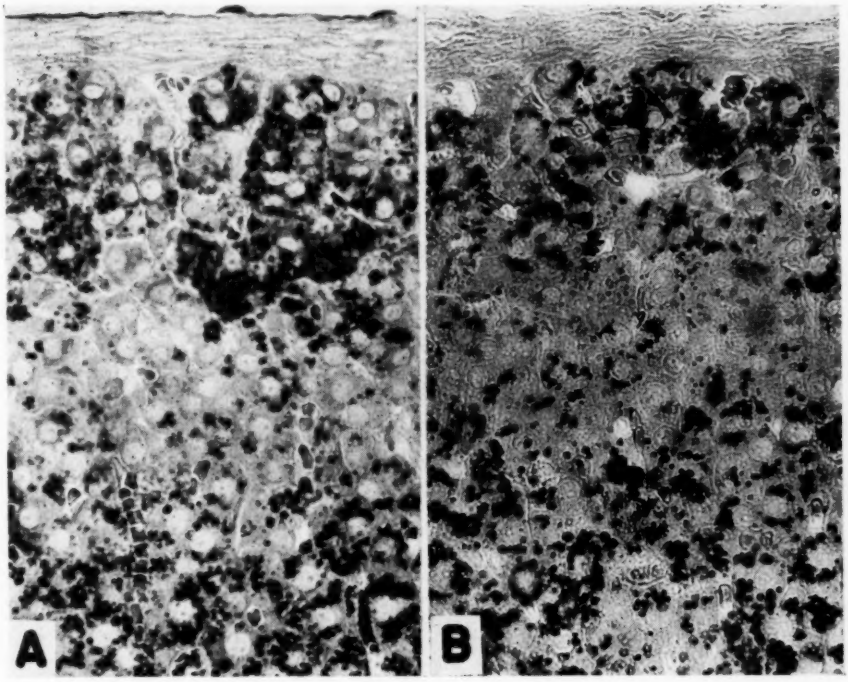

FIGURE 1.—*A*, Outer cortex of an adrenal gland of a normal adult male rat; *B*, Similar region of an adrenal from a rat killed immediately after 4 hours' exposure to a simulated altitude of 27,000 feet.

pretreatment. However, animals pretreated with ACE showed a significant increase in adrenal weight 24 hours after a single exposure, while the uninjected group showed no change. Animals not pretreated showed a significant increase in adrenal weight only after two consecutive daily exposures. ACE-injected animals that were not exposed were killed at intervals following the injection. However, all of these animals have been grouped together as ACE controls since no differences in adrenal weight or in adrenal histology which could be considered as due to the differences in times of the injections were noted in these groups.

While no change in weight was noted in the adrenals of any animals killed immediately after a single exposure, quite obvious and interesting changes in the distribution of the lipid droplets were observed. The changes seen are summarized in the last three columns of table 1. An increase in lipid is indicated by a plus sign, a decrease by a minus sign, and no change by zero. Two plus signs indicate that the lipid-free zone of the fasciculata could not be distinguished from the adjacent zones owing to the appearance of a large number of lipid droplets in this region.

Figure 1, *A*, shows the normal distribution of lipid droplets in the outer cortex of an adrenal gland of an adult male rat. The zona glomerulosa is sharply demarcated from the zona fasciculata by the cells of the lipid-free zone. The cells of this region, ordinarily included as part of the zona fasciculata, are usually smaller in size than the neighboring cells of either the zona

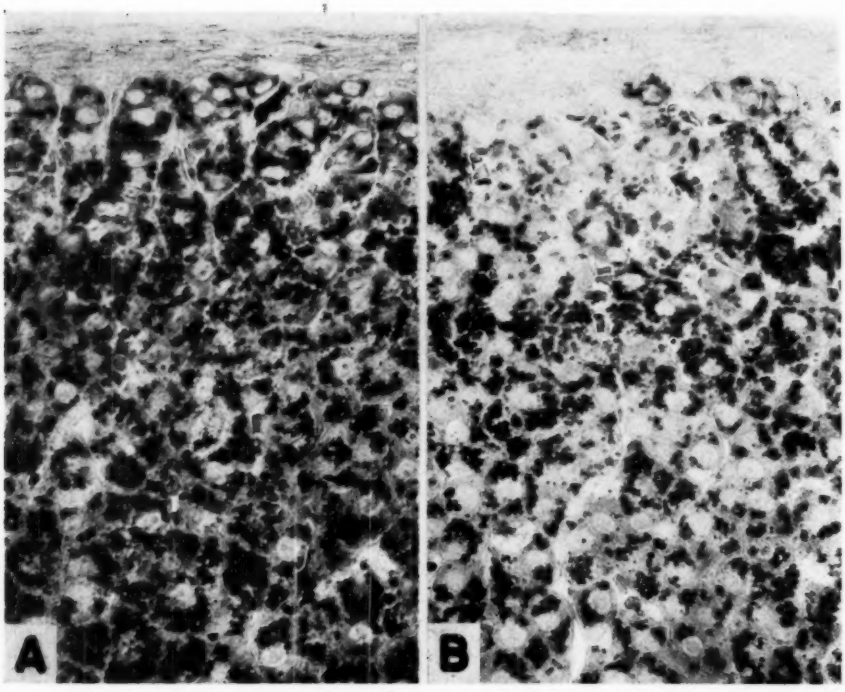

FIGURE 2.—*A*, Outer cortex of an adrenal from a rat killed 20 hours after 4 hours' exposure at 27,000 feet; *B*, Outer cortex of adrenal from a rat given the same treatment as in *A*, except that it was given 1.00 cc. of ACE 8 hours before exposure.

glomerulosa or the zona fasciculata. The majority of cells of this layer contain no lipid droplets in normal adrenals. When lipid is present, it is found in the form of small droplets clumped together in a small area of the cytoplasm.

Figure 1, *B*, shows the outer cortex of an adrenal gland of a rat exposed to a simulated altitude of 27,000 feet for 4 hours. It may be seen that the cells of the lipid-free zone contain fairly numerous, small, evenly distributed lipid droplets. This change has been found to be characteristic of the adrenals of all animals subjected to a single exposure of short duration and is the only consistent change that has been noted. However, a decrease in stainable lipid may be noted in individual instances in the inner fasciculata (table 1, last column). No changes were noted in the zona glomerulosa. Adrenals of animals killed 20 hours after exposure at 27,000 feet for 4 hours showed a further change in the lipid-free zone.

Figure 2, *A*, demonstrates that as a result of such exposure this zone can hardly be distinguished from either the glomerulosa or fasciculata. Apparently the increase in size and number of the small, evenly distributed droplets visible immediately following exposure results in the development of a lipid pattern similar to that of the cells of the inner glomerulosa and the outer fasciculata. In all cases the adrenals of animals pretreated with ACE and killed immediately after exposure show the presence of small droplets in the lipid-free zone. A fair proportion of the animals injected with ACE but not

exposed also show the presence of droplets in this zone. In addition, the injection of ACE appears to prevent the decrease in lipid content of the zona fasciculata which occurs in the majority of animals not receiving ACE pretreatment. However, ACE pretreatment does not prevent the increase in size and number of the lipid droplets in the lipid-free zone which regularly occurs during the 20-hour period following a single exposure (fig. 2, *B*).

REPEATED EXPOSURE

Two separate series of animals were used in these experiments. The first series consisted of young adult males averaging 273 gm. in weight. In the second series, started 6 weeks after the first, the rats were older and averaged 337 gm. in weight. There was considerable variation in weight within each group, yet the age within each group did not vary by more than 1 month. Previous experience indicated that within reasonable limits age and weight were of considerably less importance than the general state of health in so far as the reaction of the individual to conditions of lowered oxygen tension were concerned. Therefore, selection of the animals was made on the basis of health rather than on weight alone.

Individual body weights were recorded three times weekly throughout the exposure period. Table 2 summarizes the results on both groups. The more important changes in body weight and adrenal weight in the controls and in the combined groups are given in table 3. Figure 3 illustrates the changes in body weight and adrenal weight in both control and experimental groups. The adrenal weight in milligrams per gram of body weight was greater in all exposed animals than in any of the controls. This was true whether the original or the final body weights were used in the determinations. High adrenal-weight values were found in both groups at the end of 6 weeks' exposure. In group 1, there was evidence of a gradual decrease in weight during the last 6 weeks of exposures, the final weights, however, remained well above normal values. In group 2, the absolute adrenal weights remained high through the last 6 weeks, but in relation to body weight there was a relative decrease during the last 4 weeks of exposure.

Figure 4, *A*, is a section of an adrenal of an animal subjected to 2 weeks of repeated exposures and is typical of all adrenals of all animals exposed for 6 weeks or less. The figure shows the lipid pattern characteristic of the adrenals from animals of these groups. The lipid-free zone cannot be identified. The lipid content of the zona glomerulosa and zona fasciculata remained high throughout the exposure period, and the impression was gained that it is in gen-

TABLE 2.—*Repeated exposure of rats to lowered oxygen tension*

Exposure (in weeks)	Number of animals	Body weight		Adrenal weight			
		Original, average	Final, average	Average	Range	Per gram original body weight	Per gram final body weight
Group 1:		*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
2	12	264	237	36.6	30.0–43.6	0.139	0.154
4	12	303	275	40.5	30.8–52.4	.133	.147
6	11	263	266	52.3	43.2–92.9	.199	.196
8	12	256	249	46.7	35.3–64.5	.182	.188
10	11	245	246	40.8	35.0–47.8	.167	.166
12	12	306	290	44.8	34.0–62.2	.146	.154
Group 2:							
2	12	293	265	36.5	28.0–45.4	.125	.138
4	12	311	277	42.8	37.6–51.3	.138	.155
6	12	320	270	45.8	34.5–51.4	.143	.170
8	12	332	305	51.6	39.5–57.6	.156	.169
10	12	368	315	51.6	43.4–64.5	.138	.164
12	12	396	335	52.9	41.7–63.9	.134	.148

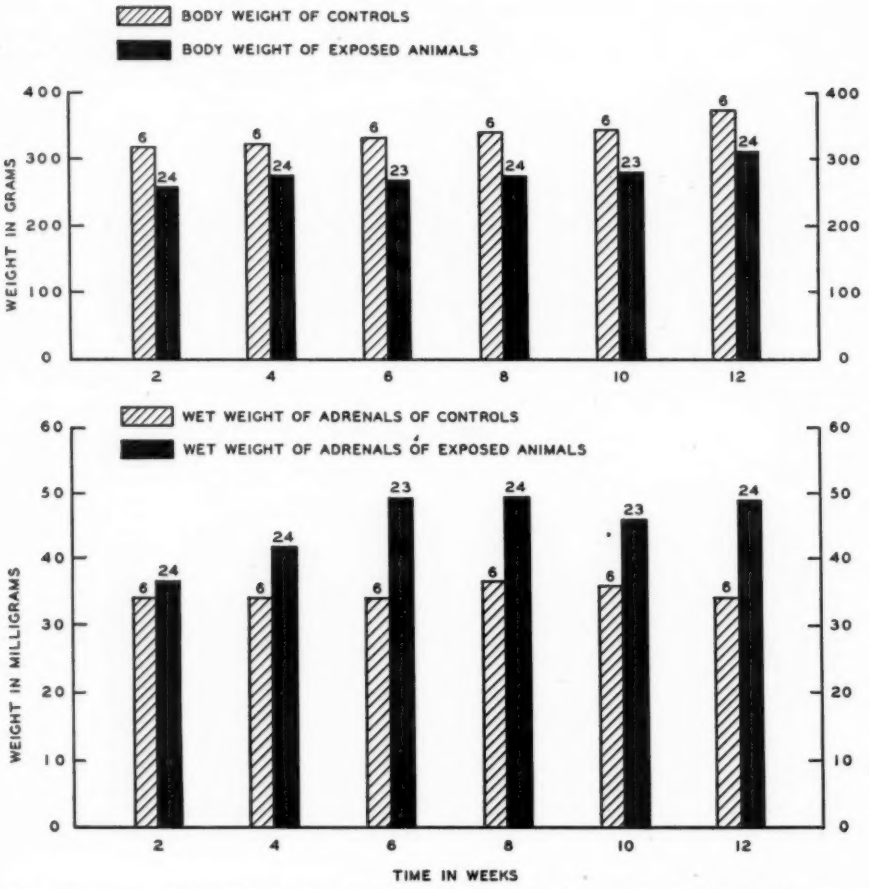

FIGURE 3.—Increase in adrenal weight following repeated daily exposures at 25,000 feet for 4 hours. The figures above the columns indicate the number of animals involved.

eral higher than in adrenals from control animals. The lipid-free zone, unidentifiable in the adrenals of animals killed during the period of increasing adrenal weight, was again found in animals killed during the last 6 weeks of exposures when the adrenal weight was remaining constant or was decreasing. Figure 4, *B*, shows the outer cortex of an adrenal gland from an animal exposed for 12 weeks. The lipid-free zone is again in evidence, and the whole cortex appears histologically normal. While the distribution of lipids is similar to that in the control animals, both the zona fasciculata and the zona reticularis appear to have increased in width. From the visual inspection of many adrenals, the impression was gained that the increase in adrenal weight during repeated exposure to anoxia is in great part the result of the increase in thickness of these two zones.

In a few instances, evidence of severe localized damage with repair and scar-tissue formation was found, but these

TABLE 3.—*Repeated exposures of two groups of rats to lowered oxygen tension*

Exposure (in weeks)	Number of animals	Final body weight		Adrenal weight		
		Average	Range	Average	Range	Per gram final body weight
Controls:		*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*
2	6	317	277–338	33.9	31.2–39.5	0.103
4	6	323	302–327	32.6	29.0–38.3	.101
6	6	330	287–443	33.8	27.6–43.7	.103
8	6	339	317–353	36.6	29.7–41.5	.108
10	6	344	308–374	36.0	30.0–43.7	.105
12	5	375	336–432	34.3	32.5–34.8	.091
Exposed:						
2	24	257	202–318	36.6	28.0–45.4	.143
4	24	276	236–306	41.7	30.8–52.4	.151
6	23	268	241–299	49.1	34.5–92.9	.183
8	24	276	193–357	49.2	35.3–64.5	.177
10	23	282	209–356	46.0	35.0–64.5	.163
12	24	313	261–394	48.9	34.0–63.9	.156

instances were scattered and showed no correlation with the number of exposures.

DISCUSSION

ACUTE EXPOSURE

The significant increase in weight 20 hours after a single 4-hour exposure to lowered oxygen tension is interesting. This result suggests that the exposure is sufficient to cause activation of the pituitary and release of adrenocorticotropic hormone. Ingle (*15*) showed that adrenal hypertrophy occurs within 12 hours after the beginning of muscular work. Exposure to lowered oxygen tension is known to cause adrenal hypertrophy, and it is not surprising that this hypertrophy occurred within the 20-hour period noted. The fact that adrenal enlargement occurred in the ACE–treated group and not in the untreated group is surprising since Ingle found that his "work induced" hypertrophy could be prevented by cortin treatment. It may be reasoned from this that the cortical extract used contained little of the substances responsible for the inhibition of the adrenocorticotropic hormone of the pituitary.

The rapid formation of lipid droplets in the lipid-free zone of the adrenals in response to anoxia is also interesting. As far as the authors know, histologic evidence of such an early response of the adrenal cortex to stimulation has not been reported previously. In the majority of our cases, it was noted that while formation of lipid droplets was occurring in the lipid-free zone, a decrease in lipid was occurring in the "secretory" portion of the zona fasciculata. If it may be assumed that these changes in the adrenal cortex are caused by the action of adrenocorticotropic hormone released from the pituitary, it would appear that cortical cells in different stages in their life cycle react in a different manner to the same primary stimulus.

Apparently the zona glomerulosa of the adrenal is not involved to any extent in the cortical changes following hypophysectomy, involution being confined to the zona fasciculata and zona reticularis (*8*). Further, the cortical atrophy resulting from desoxycorticosterone treatment in intact animals is confined to the zona glomerulosa (*8*). More complete adrenal atrophy, including changes in the zona glomerulosa, occurs after hypophysectomy plus desoxycorticosterone treatment than after hypophysectomy alone. These findings may be correlated with the results of the work of several investigators which indicate that normal carbohydrate metabolism, particularly, is interfered with in the hypophysectomized rat while the electrolyte balance remains fairly normal (*16*). This fact suggests that while the hormones concerned with carbohydrate metabolism become depleted in such animals, substances

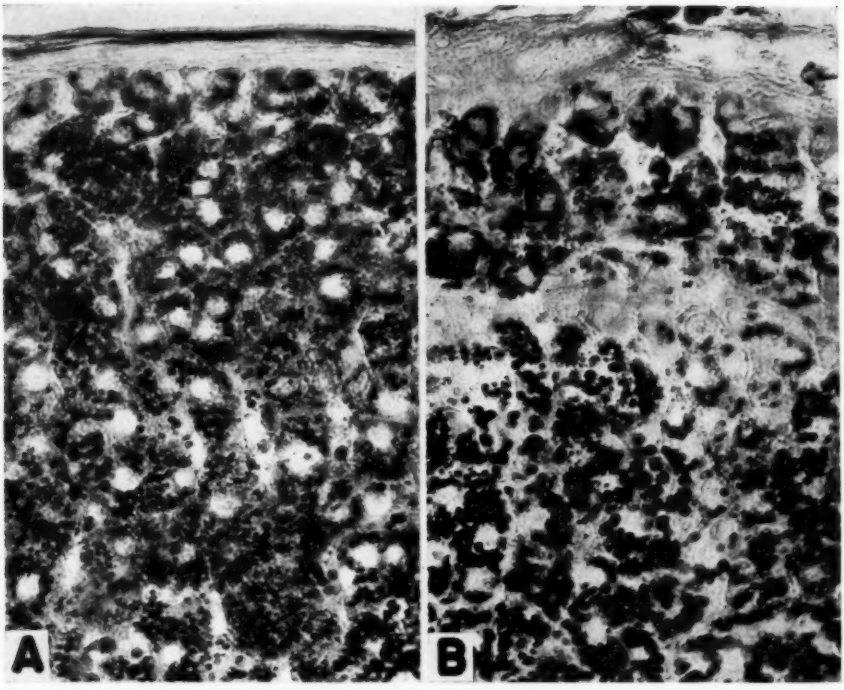

FIGURE 4.—*A*, Outer cortex of an adrenal from a rat exposed 4 hours daily to 25,000 feet altitude for 2 weeks; *B*, Outer cortex of an adrenal from a rat that received repeated exposures for 12 weeks.

having an action similar to desoxycorticosterone continue to be formed in nearly normal amounts. From the foregoing, it may be inferred that substances concerned with control of the electrolyte balance are formed in the outer cortex (glomerulosa) and that as a result of the stimulation by the pituitary, substances concerned with carbohydrate metabolism are formed in the inner cortex (fasciculata). It is a rather well established fact that new cortical cells are being formed continually by addition from cells of the inner portion of the capsule (*5*) and by mitosis in the zona glomerulosa (*3*) and that these cells migrate inward (*17*), pass through cycles of storage and secretion, and die at the medullary border.

It is our opinion that the presence of a lipid-free zone between the lipid-filled zona glomerulosa and zona fasciculata in the adrenal of the normal rat suggests that cortical cells as part of the zona glomerulosa form and store lipid material and release it as they pass into the lipid-free zone. As cells of the zona fasciculata, stimulated by the pituitary hormone, they again form and store lipid, release it in the inner fasciculata or reticularis, and finally die at the medullary border. Inherent in this view is the implication that at one stage in their history the adrenal cortical cells form but one general type of hormone and that different types of hormones with different actions are formed at different times in the life of the cells. Such a view is not un-

reasonable, in view of the similarity in the various compounds under consideration.

It is recognized that acute, marked stimulation of the adrenal cortex results in adrenal enlargement, with a loss of lipid from the inner portion of the cortex. It would also appear that mild chronic stimulation results in an accumulation of lipid owing to the fact that it is formed more rapidly than it is released. In this case there is adrenal enlargement with an increase in lipid content (*5*). Thus the lipid content of the adrenals decreases on acute stimulation but increases with moderate chronic stimulation.

In the present study, a picture comparable with that found by Zwemer (*5*) to result from mild chronic stimulation (treatment with moderate doses of anterior pituitary extract) has been found consistently through the second to the sixth week of repeated exposures. The adrenals were enlarged and contained a larger amount of stainable lipid than did normal glands. The lipid-free zone could not be identified. This picture contrasted with that seen following single acute exposure, in which there was evidence of lipid loss from the fasciculata and the presence of small, evenly distributed lipid droplets in the lipid-free zone. According to Tepperman and Engel (*16*) Sarason found the adrenals of rats depleted of lipid during the first 5 days of continuous exposure to anoxia, yet by the seventh or eighth day of exposure, the lipid pattern of the glands had reverted to normal. Van Liere and Stickney (*18*), using hemoglobin concentrations and erythrocyte levels as criteria, obtained evidence of acclimatization to lowered oxygen tension in dogs after 17 weeks of discontinuous exposure, whereas Armstrong and Heim (*13*), using rabbits, could find no evidence that discontinuous exposure would result in adaptation. Thorn, Jones, et al. (*14*) demonstrated that both rats and rabbits would become adapted to conditions of repeated daily exposure. They showed that the ensuing adrenal enlargement and thymus atrophy appeared to be related to the degree of anoxia rather than to the number of exposures. Their results agree in general with those presented here rather than with those of Armstrong and Heim (*13*). However, their experiments were not continued into the period of relatively complete adaptation (8–12 weeks). In the present series, adrenals with what could be considered a normal distribution of lipids were found in animals that had received more than 6 weeks of repeated exposures. These results suggest that acclimatization, as measured by the lipid pattern of the adrenal cortex (*7*), had been attained following approximately 6 weeks of discontinuous exposure. Evidence from the study of other organs, from hemoglobin concentrations, and erythrocyte and reticulocyte counts [3] also suggests that acclimatization was attained after this interval.

SUMMARY

Male Sprague-Dawley rats were subjected to single and repeated exposures to lowered oxygen tension. The weights of the adrenal glands of these animals were determined, and the glands were examined for changes in lipid content.

Immediately after a single 4-hour exposure, the zona fasciculata usually contained less lipid than did the same zone in normal glands. Twenty hours after such exposure, all adrenals contained less lipid than normally. The injection of adrenal cortical extract appeared to prevent this loss of lipid.

A single exposure resulted in the appearance of evenly distributed lipid droplets in the "lipid free zone" of the outer fasciculata. Twenty hours after exposure, the lipid-free zone became unidentifiable as a result of the accumulation of numerous lipid droplets in this zone. The injection of adrenal cortical extract did not prevent this change.

Repeated exposure to lowered oxygen tension caused adrenal hyperthrophy, which reached a maximum at 6 weeks.

[3] Data to be reported later.

With continued exposures, a gradual return toward the original size occurred.

With the exception of the first few days, the adrenal glands of animals exposed for less than 6 weeks were characterized by a distribution of lipids similar to that found by Zwemer following treatment with anterior pituitary extract, i. e., while the adrenals were enlarged, the amount of stainable lipid was higher than normal, and the lipid-free zone was not visible.

Animals exposed more than 6 weeks possessed adrenal glands which contained approximately normal amounts of lipid distributed in a normal pattern, including the presence of a lipid-free zone.

These changes in the adrenal glands, along with changes in other organs, suggest that adaptation to conditions of lowered oxygen tension may result from discontinuous exposure. Under our conditions, acclimatization was more or less complete after 6 weeks of repeated exposure.

The significance of the changes in the lipid-free zone in relation to the life history and functional activity of the adrenal cortical cells is discussed.

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ORIGIN OF HEPATOMA E OF STRAIN C3H MICE

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The transplantable hepatoma designated as tumor E of the strain C3H mice was derived from a primary tumor excised from the liver of a mouse on December 13, 1938. This mouse was one of seven received from Dr. H. B. Andervont early in November 1938. These mice had been given injections of 2-amino-5-azotoluene from October 1937 through October 1938. The dye had been moistened with glycerol and administered subcutaneously in 10-mg. doses in the right axilla. Of the seven mice, three, Nos. A_1, A_2, and A_3, were autopsied in November 1938, and one, No. A_4, was autopsied in December 1938. All four were found to have liver tumors. The remaining three, Nos. A_5, A_6, and A_7, which were autopsied in January 1939, had no tumors.

The initial 4 primary tumors excised from mice Nos. A_1, A_2, A_3, and A_4 were each finely minced with iris knives, suspended in isotonic saline solutions, and implanted into the axillae of mice of the same strain. The tumor of mouse A_1 was implanted in 8 mice, and those of A_2, A_3, and A_4 in 5 mice each. Of these 23 implanted mice, only 1 mouse, No. 184, which had received tissue from mouse A_4, developed a tumor at the site of implantation. From this first-passage tumor, a transplantable strain of hepatoma cells has been maintained. This strain has been carried on as strain E. From the second passage of the tumor, only 1 mouse, No. 250, produced viable transplantations in the third passage: the tumor tissue of this animal was transferred through mice Nos. 265 and 266. From the fourth passage on, the strains of hepatoma cells were readily maintained.

Table 1 gives the number of animals receiving tumor tissue, the number of positive transplants, the time required for tumor development, and a pathologic analysis of the sections of some of the tumors of each passage. The data show that there was an increase in the number of successful implantations with successive passages, which was accompanied by a distinct reduction in the time required for tumor development. In the fifth passage, the time required for tumor development was about one-third that required for tumor development in the first passage. Kinosita [1] reported the reduction in time required for development in early successive passages of hepatic carcinoma induced in rats with 2-amino-5-azotoluene. Andervont [2] obtained similar results with pulmonary tumors induced in mice with dibenzanthracene and methylcholanthrene.

Sections of the liver showing the primary tumors of mouse A_4 were described [3] as follows:

> There are numerous liver cell tumors which generally are uniform and reproduce liver architecture closely. In a few areas the cells are larger, with dark nuclei and smooth cytoplasm. In one area is a small group of irregular vascular channels filled with blood and partly lined by endothelium. Moderate cirrhosis. Diagnosis—Hepatoma. Cirrhosis of the liver.

Sections of the first transplanted hepatoma from animal 184 showed cords of liver cells resembling areas in primary growth. The tissue was not considered to be malignant and was identified as a "transplanted hepatoma in subcutaneous tissue."

In the next passage (mouse 250), sections of the tumor showed that it was growing in solid sheets in some areas and as liver cords separated by sinusoids in others. It was designated as a transplantable hepatoma.

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[3] Diagnosis of tumors furnished by Dr. H. G. Grady, of the National Cancer Institute.

TABLE 1.—*Origin and early passage of hepatoma E obtained from strain C3H mouse A_4*

Tumor passage	Animals with tumor used for transplant Nos.—	Pathologic analysis of tumor	Mice implanted	Mice obtained with tumors	Interval between transplantations	Average time required for development of tumor from transplant
			Number	*Number*	*Days*	*Days*
First, of primary tumors	A_1		8	0		143
	A_2		5	0		
	A_3		5	0		
	A_4	Hepatoma	5	1	143	
Second	184	do	5	4	167	167
Third	248	do	2	0		130
	249	do	1	0		
	250	do	2	2	130	
	251		1	0		
Fourth	265	Hepatoma	10	8	118	111
	266	do	9	5	105	
Fifth	284	do	4	0		43
	285	do	4	0		
	286	do	3	3	32	
	287	do	4	1	41	
	288	do	4	4	32	
	289	do	2	2	32	
	290	do	4	0		
	291	do	3	0		
	292		4	3	55	
	293	Hepatoma	2	0		
	136	do	4	3	66	
Sixth	303		3	3	46	49
	302		2	2	74	
	295		2	2	45	
	296	Hepatoma	2	2	38	
	319		5	5	56	
	324		4	4	35	

Sections of the third passage in mice 265 and 266 were similar to the previous passage, but with a large number of intranuclear inclusions.

The sections of all the tumors of the fourth passage were designated as hepatomas. The tissue for the most part was firm and homogeneous in appearance, with little or no necrosis. In some tumors hemorrhagic areas were present.

In the fifth passage, sections showed hepatoma cells arranged in cords separated by endothelial sinusoids.

In general it may be said that hepatoma E develops firm sheets of hepatoma cells and that the hemorrhagic areas do not appear until the tumor is old. The formation of hemorrhagic areas is usually accompanied with enlarged capillaries and irregular sinusoids. Transplantations are most successful when the tumor shows firm areas of hepatoma tissue.

Since the early passages of the tumor, this hepatoma strain has been maintained by successive transplants in C3H mice at intervals of approximately 4 weeks. It is now in the fortieth transplant generation and is palpable about 14 days after implantation. This strain of hepatoma has been issued to various laboratories for further experimental research.